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Cinderella in the UN

MANY of those in touch with the preparations for the UN Conference on Science and Technology for Development (UNCSTD), now scheduled for Vienna in August 1979, have been concerned with the apparently low priority given to possible contributions from the world scientific community. Recently, however, there would seem to have been a change of heart, if not within the UN's own Office of Science and Technology, at least on the part of Mr da Costa, the Conference's Secretary General.

This much became apparent at the meeting in Geneva last November of the Advisory Committee on the Applications of Science and Technology, ACAST, the United Nations' informal committee. ACAST was one of the few concrete results of the last big general conference on the Application of Science and Technology for the Benefit of Less-developed Areas (UNCSAT) held in 1963. Rather to the surprise of ACAST's members, who had previously found him somewhat inclined to play down the part that scientists might play in 1979, da Costa went to some pains to stress the important contribution they could and should make not only at the Conference itself, but also in the preparation of the national papers that will provide the principal input to UNCSTD. He spoke, too, of the "necessity to ensure the participation of scientists at the forthcoming session of the UNCSTD Preparatory Committee", which "will allow them to speak alongside diplomats at a very early stage in the Conference preparations".

In fact, competition for the floor of meetings is less likely to be between scientists and diplomats than between scientists and 'experts' in the development business who represent, in UN terms, the UNCTAD and UNIDO lobbies.

The UN is still agonising about whether it should have a science and technology policy at all. This is the subject of an important report from ACAST that will be considered by the Committee on Science and Technology for Development (CSTD—the secretariat of UNCSTD) at its meeting which starts on 23 January in Geneva. While ACAST itself believes that "there is a need for a policy which would guide and facilitate the development and application of science and technology by and within the United Nations system for the benefit of its Member States" the various members of the UN family, and in particular the specialised agencies, seem

decidedly lukewarm if not frankly antagonistic to this idea. The fact is that, although themselves almost without exception based to a greater or less extent on science or its immediate applications, they are notoriously jealous of their own sovereignty in matters of policy—indeed, it sometimes seems that this is almost the only thing on which they can agree.

Nonetheless, if the agencies which purport to advise and assist the developing countries in applying science and technology cannot agree at least to certain priorities in this field, much of their effort will be wasted and the developing countries themselves will still lack the guidance they so desperately need on where to go and how to set about getting there. In this context, the new report from ACAST is well worth careful study and consideration, as much outside the UN system as within.

As to ACAST itself, after a period when, again within the UN system, its very usefulness has been called in question, the recent meeting should have dispelled any doubts as to its value. Its members do not sit as official government representatives but in their own right as scientists of international repute, and covering a very wide range of disciplines. This also seems to have been recognised by Mr da Costa when he stressed the value of ACAST's role in "acting as a bridge between the Conference Secretariat and the scientific community" particularly with regard to the participation in 1979 of the major non-governmental organisations such as ICSU, the Pugwash Conference and the World Federation of Engineering Organisations (WFEO), all of whom found it worthwhile to send observers to the ACAST meeting.

One point which may be of concern to British scientists is that at present no scientists from this country is a member of ACAST. This is partly because in inviting scientists to sit on this Committee, the UN Secretary General seeks to avoid the impression of any permanent 'national' representation. In part, too, even ACAST is affected by the move to include more representation of the developing countries among its members (in fact the Chairman of the recent meeting was Dr Chagula of Tanzania). Be that as it may, there is little doubt that when, as will surely happen sooner or later, there is any suggestion that a British scientist should once again be invited to sit on this, the only UN body that can speak for science and scientists, it should be warmly welcomed. □



Is British physical anthropology dying?

Bernard Campbell, Adjunct Professor of Anthropology, University of California, Los Angeles, argues that teaching and research in physical anthropology should be developed and expanded in the UK.

RESEARCH and teaching in the most fundamental branch of the science of physical anthropology is today almost extinct in Great Britain. Senior positions are no longer available, and almost all the talented scholars in the field have moved to the US or Canada. Human palaeontology, as a key part of the anthropological sciences, is taught by permanent staff in the Anthropology Department of only one University: Cambridge. While it is also taught in a limited way in at least five anatomy departments of medical schools, this teaching is generally in the context of human anatomical studies and is therefore not in its most appropriate milieu, namely, the knowledge of human society and culture, and of the evolutionary paradigm.

This is a near disaster. Physical anthropology, concerned as it is with man's biology, is the study of the most fundamental aspects of human nature. While it can be said to date from J. F. Blumenbach (1752-1840) (who classified races and made a collection of human skulls) it came of age and gained its central paradigm with the publication of Darwin's *Origin of Species* (1859).

Today, physical or biological anthropologists study Man as a product of natural selection and try to understand human variability in the light of evolution. The biology of Man has branched into many sub-disciplines, including comparative anatomy and comparative physiology, which are at the centre of human biology; the study of human growth and variability; genetics; primate palaeontology; primate behaviour; human ecology; and many others. Physical anthropology is therefore necessarily disparate in its specialisations.

At University College, London, for example, staff are presently doing research into the systematics of the *Galagidae*, the genetics of marriage in modern Britain, the serological genetics and nutrition of Carib populations, the genetics of Ethiopian baboons, and the strength and structure of bone. Many of these specialisations lean heavily on developments in related sciences, especially human genetics and physiology, and zoology. Sometimes it seems that these diverse specialists would be better placed in other departments. Yet research into human blood-groups was immensely stimulated by anthropological considerations and data, and studies of primate behaviour were given a new focus by anthropologists S. L. Washburn and I. DeVore. Anthropologists have both received from, and given to, related disciplines, and their central interest in 'the proper study of mankind' energises and directs their research.

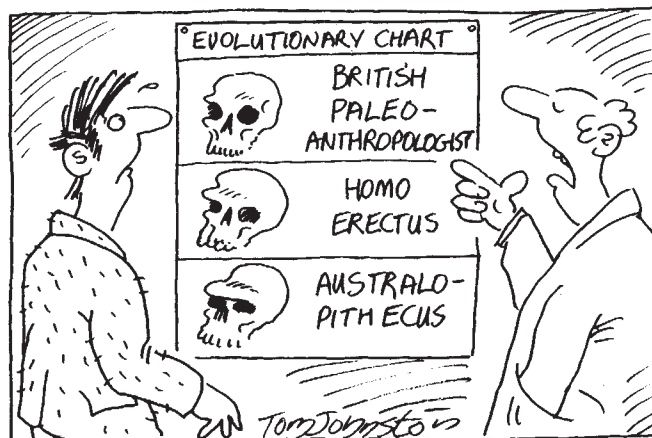
In the early years of this century Karl Pearson, G. M. Morant and others, worked painstakingly with calipers and measuring tapes to produce a vast amount of quantitative data about the skeleton of modern man. They also studied the rather limited fossil record in the same way. The method produced meagre results and in the 1940s Fisher's population genetics and the new research on the ABO blood groups developed as a far more powerful tool with which the nature of racial differences and at least recent human prehistory might be uncovered. The dimension of time proved elusive, however, and while the genetical aspects of Man's place in nature probably hold pride of place today in most departments of physical anthropology throughout the world, these studies do not and cannot develop that central explanatory paradigm, which alone can hold physical anthropology together as a science.

The fossil evidence for human evolution and its interpre-

tation into a more complete understanding of Man's past has become alive again with the development of a functional approach to anatomical analysis. S. L. Washburn was probably the first to insist on its importance (circa 1950) and others followed. My book *Human Evolution* (1966) was an early attempt to make such a functional integration. The bones came to life, and behavioural speculation followed. As a result physical anthropology came closer to its sister discipline, prehistoric archaeology: together they constitute the science of evolutionary anthropology or palaeoanthropology *sensu lato*. Palaeoanthropologists are not today expected to be outstanding scholars in both human palaeontology and prehistoric archaeology, but they are expected to be very much aware of the value of each science and its vital importance in understanding the evolution of human nature. The interdisciplinary study of the evolution of man which also includes vertebrate palaeontology, palaeoecology, and palaeogeography, is necessarily the main stem from which all the sub-disciplines of physical anthropology mentioned above are nourished. In spite of the complexity of anthropology, an anthropologist must necessarily be a generalist as well as a specialist: he must specialise in the light of his knowledge of mankind's whole adaptive strategy.

Human palaeontologists must, therefore, more than ever before, rub shoulders with social and cultural anthropologists and archaeologists; and at the same time they must see man at all times—not as a creature apart from nature, but as a very remarkable animal. It follows that although the anatomy departments have housed and nourished human palaeontologists for three to four generations in the UK (since the time of Sir Grafton Elliot Smith and Sir Arthur Keith), they can no longer remain the primary resource for this subject.

At a recent inaugural meeting in London, the L. S. B. Leakey Foundation presented its goal of further study into "Man's origin, behaviour and survival", and plans to support students and research projects in areas which reflect this holistic approach to the study of man. What is needed in Britain is a Department of Physical Anthropology with the prestige, money and manpower to bring back to Britain some of those palaeoanthropologists who have left. Britain needs a university with the will and the resources to bring to life the main stem of all anthropology—the study of human evolution. Man has been part of Nature—as a hunter and gatherer—for 99% of the approximately 2 million years of his existence on earth. Man is a product of his prehistory, and our understanding of our present predicament depends squarely on our knowledge of our own past. □



"Of course, they're extinct now too!"

Research with prisoners

After 18 months' deliberation the US Secretary of Health has responded to a report questioning the ethics of research involving prisoners. **Michael S. Yesley** and **Barbara Mishkin**, closely involved in the production of the report, here discuss their findings

UNDER American law new drugs must undergo three phases of testing before being approved by the Food and Drug Administration (FDA) for general sale. Phase 1 involves the first introduction of a drug in humans and requires testing on normal volunteers to determine the safety and general metabolic properties of the drug. Phase 2 consists of controlled clinical studies on a small number of patients to determine therapeutic efficacy. (In the United Kingdom and elsewhere, phases 1 and 2 are consolidated by testing for safety as well as efficacy on a small number of patients for whose treatment the drug is intended.) Phase 3 is similar to phase 2 but involves considerably more patients. Although no data are available by which to ascertain the extent of the use of prisoners in phase 1 tests in the United States, it is clear that they are used to a significant degree.

The reasons for the practice are partly historical. During the Second World War, civil prisoners in Illinois and New Jersey participated in research to develop treatment for malaria and other diseases that afflicted the armed forces. Such participation was considered not only acceptable but praiseworthy, for it gave inmates an opportunity to contribute to society (a form of restitution) and to enhance the war effort. Following the war, the involvement of prisoners in non-therapeutic biomedical research increased as a result of expanding government support for biomedical research and more stringent federal requirements for evaluating the safety of new drugs.

At the same time that the use of prisoners was increasing in the United States, the world was reacting to revelations of the experiments conducted in the Nazi prison camps. The Public Health Council of the Netherlands, for example, responded by specifically disapproving research involving children, old people, the insane and prisoners. The Nuremberg Code, initially drafted by an American consultant to the war crimes tribunal, is somewhat ambiguous with regard to the involvement of prisoners in research. The first principle of the Code provides that: "The voluntary consent of the human subject is essential. This means that the person involved should have legal capacity to give consent: should be so situated as



Self-confidence training at Wisconsin State Reformatory

to be able to exercise free power of choice without the intervention of any element of force, fraud, deceit, duress, overreaching, or other ulterior form of constraint or coercion. . . "

Many have interpreted this provision to mean that civil prisoners may not participate as research subjects, because they are not "so situated as to be able to exercise free power of choice." In the United States, however, it has been argued by the research community and by some prisoners themselves that so long as the prisoners are fully informed and no obvious duress is imposed, they may be considered free volunteers. This assertion is at the centre of the recent controversy in the United States over the participation of prisoners in research.

Jessica Mitford's book *Kind and*

Usual Punishment, published in 1973, was partly responsible for calling the public's attention to this issue. Mitford perceived drug research to be an exploitation precipitated by FDA drug-testing requirements and perpetuated by the economic self-interest of drug firms, investigators, prison authorities and the inmates themselves. Concurrently, academicians and civil rights organisations began to question the validity of any consent given by prisoners to participate in nontherapeutic research. They suggested that voluntary consent was impossible in penal institutions because of the poor living conditions, fear of physical violence in the cell-block, absence of good medical care and alternative ways to earn money in prison, and the obvious advantages, therefore, of leaving the general prison quarters to be paid for living in a research unit with access to individuals from the outside world. They also suggested that in a closed society such as a prison, the opportunity for exploitation is especially great when, as Mitford pointed out, prisoners are frequently prevented from reporting abuses to those on the outside.

Proponents of prison research have argued, on the other hand, that if research in prisons were to be prohibited, the result would be an inability to assure the safety of new pharmaceuticals, since prisoners are most likely to have the time and inclination to subject themselves to the kind of tests required by FDA to establish safety of new drugs. The proponents state, in addition, that phase 1 drug testing presents little risk, that injuries have been few, and that procedures are available to assure that consent is freely and knowledgeably given without undue influence or coercion.

In the midst of this debate, the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research was established by Congress to conduct studies and make recommendations in several areas of controversy involving

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human subjects, including the participation of prisoners. The Commission set out in 1975 to investigate the validity of the various claims put forth and to examine the legal and ethical issues surrounding the participation of prisoners in research. The members of the Commission and its staff visited several prisons where research is conducted and interviewed over 250 prisoners who participate in research. The Commission conducted public hearings at which arguments for and against prison research were presented, and sponsored a conference at which the view points of minority populations were expressed. In addition, several papers and studies were prepared for the Commission on topics including the nature and extent of prisoner involvement in research in U.S. prisons; alternatives to the use of prisoners and foreign practices in drug testing; philosophical, legal and sociological perspectives on the involvement of prisoners in research; experimental behavioural practices involving prisoners; and a sociological study of research at five prisons.

The Commission found that virtually all prisoners participating in biomedical research feel that they do so freely and do not want to lose this opportunity. Most prisoners who were interviewed candidly cited financial reward as the main reason for their participation in research, and they did not appear to be motivated by the expectations of early release (which, in fact, is not granted for research participation). Further, data indicate that even in prisons where the inmate population is predominantly black, the research subjects are predominantly white, and they tend to be better educated and to hold better prison jobs than the nonparticipants. This finding contradicts the general belief that prison research exploits minority groups and those unable to obtain good prison jobs (to the extent such employment is available).

The Commission was impressed with reports of programmes that successfully use nonprisoner volunteers in phase I drug testing. At least one principal investigator (who formerly used prisoners for such research) reported that nonprisoner volunteers are preferable for scientific as well as social and ethical reasons. It is easier to control the subjects' abuse of drugs, for example, in a well-run research facility than in a prison. Further, he reported, the subjects become experienced in the bargaining and consent process, they are knowledgeable about the responsibilities and risks they are undertaking, and it is possible to provide commercial insurance to compensate them for any injuries that might occur.

With respect to the risks of phase I



Learning to concentrate by crawling

drug testing, it appears that they are not significant. One commercial company that provides insurance for nonprison volunteers has estimated that the risk of such "employment" is roughly equivalent to that of being a secretary.

This situation, in which the prisoners expressed their desire to participate in research, while advocates and other outside observers argued that the prisoners were unduly influenced, posed a dilemma for the members of the Commission. The irony was not lost that recognition of the limited options in prison life might be used to justify the removal of one of those options. On the other hand, it is likely that many prisoners who participate in research would choose not to do so if the conditions of prison life were substantially improved or there were optional sources of equivalent income. This likelihood may indicate undue influence, and thus involvement of prisoners in research may well constitute an exploitation.

Despite the confidence of prisoners that their participation in research was entirely voluntary, the Commissioners expressed doubts that conditions generally prevalent in United States prisons provide a setting in which voluntariness can be relied upon. The Commissioners also expressed reservations about the

conduct of research in institutions that are isolated from public scrutiny and in which access to the outside is limited. The Commission concluded that the conduct of nontherapeutic research should be permitted only under acceptable prison conditions and that the burden of proof as to the social necessity of using prisoners as subjects in nontherapeutic research should be on those who wish to continue the practice.

The Commission therefore recommended to the Secretary of Health, Education, and Welfare and to Congress that the participation of prisoners in nontherapeutic research not be permitted unless any prison proposed as a site for such research is able to meet a long list of specific standards regarding health and safety conditions, job opportunities, free communication with persons on the outside, prisoner representation on research review boards, and the right of prisoners to form effective grievance committees. In addition, any investigator proposing to use prisoners in such a facility must demonstrate that the need to use prisoners in a particular research project is compelling and that such use would not result in social inequity.

The Commission recommended, however, that research designed to improve understanding about the causes of deviant behaviour, the effects of incarceration, and the factors related to parole performance and recidivism be permitted, so long as certain procedures are followed to protect the rights of prisoners, to safeguard their privacy and to preserve the confidentiality of the data obtained.

The Commission's recommendations* were submitted to the Secretary of Health, Education and Welfare in October 1976. After a delay occasioned by disagreement in the department over the wisdom of implementing the recommendations, Secretary Joseph Califano, Jr., announced last month that he would issue regulations even stricter than those recommended by the Commission for nontherapeutic research with more than minimal risk (e.g., phase I drug testing). In fact, he proposed to ban the participation of prisoners in such research under any conditions. The Secretary's stated grounds for his decision espoused the arguments of civil rights advocates that prisoners, by the nature of their situation, are incapable of giving informed consent. □

*Copies of the Commission's report and recommendations may be obtained by writing to: Public Information Officer, National Commission for the Protection of Human Subjects, Room 125, 5333 Westbard Avenue, Bethesda, Maryland 20016.

Fermilab's director threatens resignation



FOR a considerable number of years, Europe's high energy physicists have looked enviously at the facilities and resources available to their US colleagues. Recently, however, the pendulum has begun to swing the other way; American physicists, faced with rising costs and conflicting demands on a limited budget, now speak of the need to "keep up with the Europeans".

The most dramatic manifestation of this has been the suggestion by Dr Robert R Wilson, director of the Fermi National Accelerator Laboratory near Chicago, that he will resign his post next month unless the Government provides sufficient funds for early completion of the laboratory's proposed 'energy doubler', a development which would both cut down running costs and allow the laboratory to reach energies of 100 GeV or more.

Wilson stated his position in a letter to Mr James R Schlesinger, Secretary for Energy, whose department is responsible for high energy physics laboratories. Schlesinger's proposed budget for 1979 will be presented to Congress next week by President Carter. And although regarded by some as an attempt at special pleading, Wilson's move has highlighted tensions over the allocation of funds within the high energy physics community.

Fermilab—the "jewel in our crown" as one member of this community puts it—was completed on time and within budget (\$340 million) in 1972. Although designed to carry out experiments at 200 GeV, it has been operating at energies of up to 500 GeV, and was for several years the most powerful particle accelerator in the world. Much important and exciting physics has come out of the laboratory. Only last year, for example, Fermilab announced the dis-

covery of the new ψ particle of mass about 10 GeV. This particle is believed to be similar to the J/ψ at 3.1 GeV and to herald the presence of a new quark.

Yet it has been estimated that the new SPS proton synchrotron facility at the European Centre for Nuclear Research (CERN) in Geneva, which came into operation at the beginning of last year and currently operates at about the same energy at Fermilab, enjoys almost twice the level of support from research teams financed by the different European countries involved. As a recent article in the Fermilab house journal put it "whereas we welcome the arrival of our friends and co-investigators in the field of 400-GeV physics, we also must be mindful of the competition. There is no point in doing an experiment at Fermilab, quite similar to one being done at CERN, and doing it less well."

"At present, we are doing only about half the amount of physics a year that we could be doing" according to Dr Edwin L. Goldwasser, deputy director of the laboratory. "And in two or three years time, if there is no improvement in funding, we could find ourselves in a relatively weak position internationally." Already it is planned that Fermilab's meson facility is to close for six months for lack of funds.

Saving energy—and thus reducing Fermilab's \$5 million electricity bill—is therefore one of the attractions of the "doubler" project, under which a second ring of superconducting magnets would be constructed using the same tunnel as the existing ring.

According to Dr Goldwasser, a whole new set of experiments would be opened up by such a facility, which would be able to provide proton energies on a target of up to 1000 GeV (43 GeV in the centre of mass). And by circulating particles in opposite directions in the two rings—for example protons and antiprotons—collision energies of 2000 GeV in the centre of mass, at high luminosity, could be reached.

With the major part of the development work carried out, Wilson is now keen to move forward rapidly to the construction phase. "At present the doubler would cost between \$30 and \$35 million, and given this money—which would mean a total budget for the laboratory of about \$120 million in 1979—we believe that the doubler could be completed by the end of 1979" says Dr Goldwasser.

Members of the Department of Energy's High Energy Physics Advisory

Panel, who would recommend the funding, are sympathetic to Wilson's position. There is a widespread feeling that, despite new facilities and continuously increasing budgets, the overall position of high energy physics is precarious. According to Professor Sidney Drell, for example, who is chairman of HEPAP and deputy director of the Stanford Linear Accelerator Center (SLAC) in California, the present level of funding is "perilously low", with virtually all major equipment being underutilised.

In this climate, there is a natural concern that Fermilab should continue to operate effectively and competitively. And HEPAP has suggested that, for the time being at least, the administration should hedge its bets and continue to support all its major accelerator facilities. But the question has become one of deciding relative priorities.

In particular HEPAP has been faced with the choice between upgrading Fermilab's facilities by constructing the "doubler" as Wilson suggests, and providing support for a new facility, the 400 GeV intersecting proton beams project (Isabelle) at the Brookhaven National Laboratory on New York's Long Island.

At present, while emphasising that it is "strongly committed" to both projects, HEPAP's vote for top priority has gone to Isabelle, just as five years ago it recommended that funds for other areas of high energy physics be held back to allow Fermilab to be developed.

HEPAP's position was confirmed at a meeting of the panel attended by both Wilson and Goldwasser in early December (and planned, ironically, to coincide with the opening of a new gallery devoted to particle accelerators in the Smithsonian's Museum of History and Technology, with Fermilab as one of the centre-pieces).

This position is likely to be reflected in the proposed science budget for 1979 which President Carter will offer to Congress next week. In this it seems likely that a major sum will be allocated for Isabelle under the Department of Energy's construction programme, but that the sum earmarked for Fermilab's energy doubler will not be large, and will come from the laboratory's operating budget.

Wilson reaches retirement age next year, and would naturally like to see the doubler well under way—if not finished—before he goes. If the administration's proposed budget does not provide the money for him to do it, then his last chance would rest with the appropriation and authorisation committees in Congress which could—if so persuaded—add an additional earmarked sum.

David Dickson

Mediterranean pollution: one man's optimism

THE 1978 Monaco meeting of the 17 Mediterranean countries involved in the 'Mediterranean Action Plan' to clean up pollution closed last Saturday to such headlines as 'Deadlock over Med' and 'Pollution talks go into stagnation'. But in Geneva, the headquarters of the United Nations Environment Project's 'Regional Seas' programme, one man remains unrepentantly optimistic: the director of the programme, and inspiration of the Mediterranean Action Plan, Dr Stjepan Keckes.

Dr Keckes (his name is pronounced 'Keshkesh') is almost literally the power behind the Mediterranean plan. A 46-year-old marine scientist, member of the Hungarian minority of Yugoslavia, he has in 4 years established a ring of 79 cooperating research centres (some of them in perpetually antagonistic states such as Greece and Turkey, Egypt and Israel) and produced a baseline study of Mediterranean pollution which all governments respect—no mean achievement.

And with his principal colleagues Patricia Bliss (legal adviser) and Mohammed Tangi (environmental management) Keckes has been responsible for a sequence of inter-governmental agreements on pollution which, despite Monaco, has been one of the jewels in the UN's crown. Next month the first of two protocols to control dumping and environmental accidents come into force, ratified by six Mediterranean states (Spain, France, Yugoslavia, Tunisia, Lebanon and Monaco). Few international agreements move so fast: only 10% of the signatories to the 1973 London Dumping Convention, for

example, have ratified the Convention. Keckes can claim 33% in two years for the protocols of the 1976 Barcelona Convention on Mediterranean pollution. Keckes has a right to be optimistic.

Speaking this week from Geneva Keckes told *Nature* that he was "perfectly satisfied" with the meeting in Monaco, and that it had "fulfilled our expectations completely". That cannot be entirely accurate, however, for Keckes, interviewed by *Nature* in December, spoke of the Monaco meeting paving the way for the adoption of a third protocol—the one that bites, on land-based sources, which deliver 80% of the pollution load—"by the autumn" of 1978. At Monaco, however, there was little progress on the protocol and a further discussion session has been planned for Geneva in October—to be followed by a meeting in Athens to adopt the protocol at no specified date. Keckes, ever optimistic, is delighted that at least the place for the adoption of the protocol—Athens—has been decided.

The third protocol defines a 'black list' of substances that must on no account be released to the Mediterranean, and a 'grey list' which can be released but only in certain limited amounts. The governments involved have estimated that to implement the necessary controls would cost some \$10 billion in initial capital investment, a large part of it to control the industrial pollution flowing from the rivers Rhone (France) and Po (Italy). Difficulties have arisen over the North-South dialogue, with Southern Mediterranean states fearing that the con-



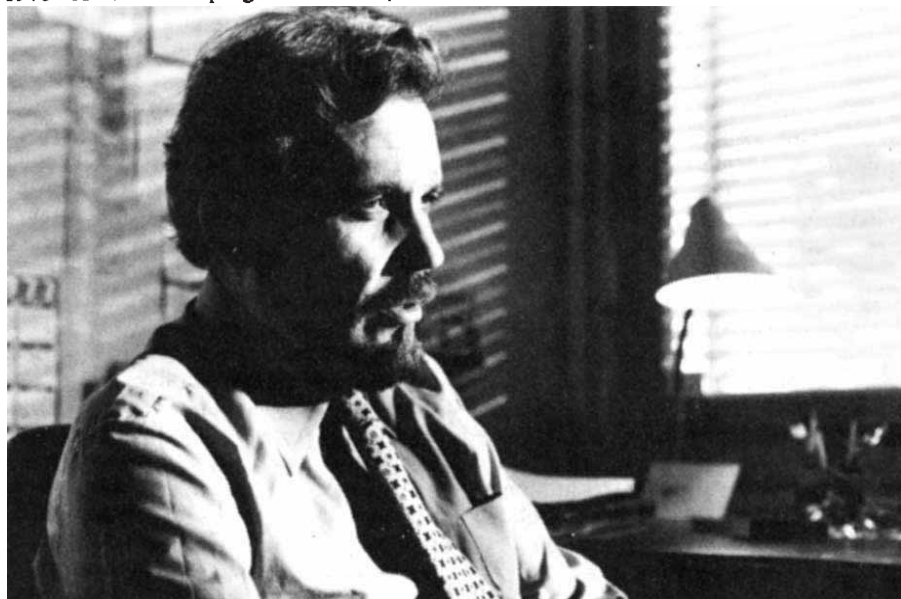
trols will inhibit their industrial development; and over whether the countries responsible for most of the pollution should pay compensation to the other states; and over the control of pollution in rivers.

Keckes remains satisfied that these difficulties will be solved, while recognising that agreeing the third protocol will be "the most difficult thing Mediterranean states will ever negotiate. We are not rushing them".

There were successes at Monaco. The most significant scientifically is the agreement to set up a study of, and consider a protocol on, air pollution over the Mediterranean. Air pollution will be "one of the biggest surprises in the future" Keckes believes. Much of the mercury and lead pollution arriving in the Mediterranean may travel first through the air, not water. And Keckes can now begin setting up some 10 to 15 new laboratories to monitor the air transport of pollutants. He expects results in 4 to 5 years. Another Monaco success for Keckes is his successful resistance of moves to make the pollution research reports secret. Keckes insisted they will be published; and they will be.

Keckes has ensured that governments approve their own research stations, so their results cannot be dismissed; and has insisted that the research be done by them rather than for them, to build up a national base for pollution monitoring. (UNEP has provided \$1.7 million for this purpose.) He has made sure that all results are intercalibrated, to be strictly comparable. And no doubt to the comfort of the least developed nations of the Mediterranean he also argues that for UNEP "protection of the environment is just one dimension of the development of the environment—for us protection for its own sake doesn't exist."

Robert Walgate



Stjepan Keckes: Confronting governments with reality

Rogers suggests interim legislation on DNA research

THE draft of a new bill covering both public and private research on recombinant DNA has been produced by Representative Paul G. Rogers in an attempt to break the deadlock on congressional legislation that developed late last autumn.

According to the provisions of the proposed bill, interim legislation would come into effect for a period of two years under which all such research would be required to be covered by guidelines laid down by the National Institutes of Health.

In addition, a commission for the study of research and technology would be set up charged with conducting a study of federal policy regarding activities involving the genetic modification of organisms and viruses. The commission would make a report within two years containing recommendations regarding federal action to be taken to promote, regulate or review such activities.

Mr Rogers is chairman of the subcommittee on health and the environment of the House Committee on Interstate and Foreign Commerce. Discussion on an earlier bill supported by the subcommittee was not completed by the full committee in the last legislative session, and the delay meant that the bill did not reach the floor of the house.

Under the provisions of the new bill, all those in the process of carrying out experiments involving recombinant DNA or proposing to do so would be required to provide an assurance to the secretary of the Department of Health, Education and Welfare that the work was being carried out in accordance with the NIH guidelines (the present guidelines, which were introduced in 1976, are currently being revised).

Those found to be ignoring the guidelines would be liable to a civil penalty (a fine) and the possible suspension of research funds.

Supporters of the new bill are hoping that by avoiding some of the more controversial aspects of the present debate—such as the problem of the 'prior disclosure' of research results, or the legal protection of employees who provide evidence of infringements—it will bring together house, senate and administration thinking, and have a higher chance of success than last autumn's attempt.

A further controversial issue is whether the federal government should be allowed to pre-empt local legislation. The bill's final position on this is likely to be the outcome of lengthy discussion with members of Congress and the administration.

But even if this approach fails to break the current deadlock, there is still a chance that the administration could develop a regulatory framework for both publicly and privately funded recombinant DNA research through existing legislation, namely section 361 of the Public Health Act.

Although the government had previously stated, following a petition from the Environmental Protection Fund in 1976, that it felt this legislation did not cover DNA research, recent re-examination of previous issues to which the act has been applied has indicated that this may indeed be possible.

Speaking at hearings held in

Washington last November by Senator Adlai Stevenson, Dr Gilbert Omenn of the President's Office of Science and Technology Policy said that the administration had rejected section 361 as being neither appropriate nor sufficient for recombinant DNA research.

However after looking at some of the previous applications more closely, the administration is beginning to have second thoughts, and is now looking closely at ways in which, if congress fails to come up with the appropriate legislation, regulations covering research with recombinant DNA could be implemented under section 361.

David Dickson

Tsiolkovskii's dream

THE link-up of Salyut-6 and Soyuz-26 and 27 marks a further step towards Tsiolkovskii's dream of a permanent orbital space station. Yet, rather strangely, the link-up was not used to effect a change of personnel, but merely to simulate one; the second crew—Vladimir Dzhanibekov and Oleg Makarov—returning to earth only five days after launch, and leaving the veterans—Yurii Romanenko and Georgii Grechko—still in orbit.

Salyut-6, with its two docking bays, represents a new generation of Soviet spacecraft. The docking bays are interchangeable, so that when Soyuz-25 failed to dock and it was suspected that there was some fault in the docking equipment, Soyuz-26, as back-up mission, docked in the second bay. Only after Romanenko and Grechko had checked out the first bay was this used for the linkup of Soyuz-27. Although there was no actual change of crew, the returning cosmonauts did change spacecraft, returning to earth aboard Soyuz-26. The launch of the second crew one month to the day after the first may be some indication of the shift-length planned for a fully operational space station; such a station, it is stressed, could save considerably on the time at present spent in warming up and mothballing the station at the beginning and end of singleton missions.

In addition to comforts for the "resident" crew—books, mail, newspapers—and the cherry juice required for the ritual toast—the "relieving" Soyuz-27 carried two temperature-stable "Bioterm-8" containers containing, respectively, the paramecium and proteus cultures for the Franco-Soviet "Cytos" experiment, which studies the effect of space-flight on cell-division. The "visiting" crew also

carried out a study of blood circulation, data from which, it is hoped, can be used to develop prophylactic measures to help cosmonauts adapt to weightlessness. Dzhanibekov, an expert on radioelectronics, checked out the various on-board systems, and all four took part in the mechanical "resonance" experiment to determine the exact strength characteristic of the compound structure.

With mail delivered again to orbit (the first time since the Soyuz 4/5 linkup of 1969), a radiorepair man on call, and (according to veteran cosmonaut Valerii Kubasov) plans for refuelling the correction engines already under discussion, Tsiolkovskii's vision seems perceptibly nearer. Yet in one respect, Salyut-6 would seem to represent a retrograde step. Although, according to Grechko, the work aboard Salyut-6 is more interesting than his previous mission on Salyut-4, since the cosmonauts can now go for spacewalks, nevertheless he misses the "oasis, where we grow plants and peas" of the earlier craft.

Vera Rich

US industry attacks Ames test

SHORT-TERM bacterial tests for potential carcinogens, such as that developed by Dr Bruce Ames at the University of California, do not predict similar responses in humans, according to the American Industrial Health Council, a New York based lobby group for the US chemical industry.

In comments on legislation covering the industrial use of potential and known carcinogens proposed by the Occupational Safety and Health Administration, the council, whose members include representatives of the

major US chemical companies, says that while such tests have value as screening tools, they are too unreliable as predictors of human responses to be sufficient to warrant regulatory action.

The council recommends that emphasis should be placed both on data from animals exposed to low levels of potential carcinogens over normal life-spans, and where prior worker exposure has occurred, on human epidemiological data. It rejects the notion that it is realistic to impose a "no risk" approach to potential carcinogens, and suggests rather the development of

dose-risk data, using risk/benefit analysis to determine acceptable-risk levels of exposure.

The council also suggests that the National Academy of Sciences should set up a classification panel to examine the hazards of potential and known carcinogens, which would be responsible both for proper category assignments and for the modification of categories as relevant advances in science dictate.

AIHC believes that setting up such a panel would provide some degree of insulation from a wide variety of

"political and other pressures" to which regulatory agencies are subject, requiring such agencies to make politically-acceptable decisions and to pay "somewhat less attention" to their scientific basis.

"The classification of carcinogens is a scientific, not a regulatory question, and would be much better handled by an independent group of scientific people than by a regulatory agency" according to Dr Ellwood P. Blanchard of the Dupont Company, a member of the AIHC steering committee.

David Dickson

Dioxin meeting recommends cancer study

SINCE the release of dioxin (the isomer 2, 3, 7, 8 tetrochloro dibenzo-p-dioxin) over a populated area at Seveso in Italy on 10 July 1976, there has been fear that the chemical is a carcinogen. Few data have been available, but it is beginning to seem that the fear is justified; hence the meeting in Lyons last week hosted by the International Agency for Research in Cancer (IARC) and the US National Institute of Environmental Health Sciences. The conclusion of the meeting was that a major epidemiological survey should be established.

It is now known that there have been at least 14 incidents in different chemical plants throughout the world where workers have been exposed to chlorinated dibenzo dioxin. In two cases the public has been exposed: at Seveso and in the herbicide spraying programme in Vietnam. In the latter cases large populations were exposed to low levels of dioxin; but while these populations must be monitored to assess the public health risk associated with low level exposure, at Lyons the opinion was that little could be learned from them. If dioxin exposure was to be unambiguously related to clinical and pathological findings, the meeting argued, the chemical plant workers were the group to monitor.

Three of the industrial accidents discussed in Lyons occurred between 20 and 30 years ago. Evidence is available from one of these accidents to suggest that in recent years there has been a marked increase in certain types of carcinoma in workers exposed to dioxins.

However the importance of this finding is marred by the small numbers of workers monitored. The meeting considered that it was therefore an urgent matter for a larger population to be studied to detect trends in carcinoma incidence which would be statistically significant. It urged the

chemical companies concerned to make their records available for scrutiny. And the meeting stated in no uncertain terms that all the workers exposed to dioxin—whether they are still employed by companies or employed elsewhere—must be located. Mortality trends for this population must be collected.

Two groups reported animal studies to test the carcinogenicity of dioxin: the first directed by Dr James Allen at the University of Wisconsin, and the second by the Doll Chemical Company. Both research teams identified the dioxin as a carcinogen. But the Wisconsin team claims that the chemical is carcinogenic at a concentration 700 times lower than the level that produce tumours in Doll's study. Reasons for the discrepancy are still not clear, but it was pointed out at the meeting that the Wisconsin study is based on an extremely low number of animals.

Some of the companies involved in dioxin accidents fear further independent investigation, not wishing to run the risk of vast compensation claims. And the meeting felt that, if companies made it a condition that their participation in a dioxin workers' survey were to be treated in confidence, this wish must be respected. The view was that, wherever the blame lies, the most important task at the moment is the compilation of information; and this the IARC is willing to undertake. Dr Rodolfo Saracci of the Unit of Immunology and Bio-Statistics agreed to direct some of this work.

The IARC will act as a clearing house for data on chlorinated dibenzo dioxin—with the help of a permanent secretariat co-opted from participants at the meeting. One of its first tasks will be the production of a list of recommended clinical procedures for examining people exposed to dioxin. It will be stressed that where possible the

clinical findings should be related to body dioxin levels—the 'body burden'. This information together with the mortality data should leave the IARC better able to assess the danger to health caused by exposure to dioxins; cancer may be only one of the possible risks.

Alistair Hay

NASA chooses its space lab candidates

FOLLOWING a similar announcement from the European Space Agency (*Nature*, January 5), the US National Aeronautics and Space Administration has announced the six American scientists from whom one will be selected as a payload specialist on the first flight of Spacelab, due to be flown in NASA's Space Shuttle in 1980.

The names of the finalists are:

Dr Craig L. Fischer (40), of the Palm Desert Medical Group Inc, California; Dr Michael L. Lampton (36), of the University of California, Berkeley; Byron K. Lichtenberg (29), Massachusetts Institute of Technology; Dr Robert T. Menzies (34), NASA Jet Propulsion Laboratory, Pasadena, California; Ann F. Whittaker (38), NASA, Marshall Space Flight Center, Huntsville; and Dr Richard J. Terrile (26), California Institute of Technology, Pasadena.

Five of the ten candidates that have now been named by NASA and ESA will undergo extensive training following a final selection in early spring. Of these, two will go into space, while the other three will perform support and advisory roles in the control centre on earth. □

Government accuses nuclear industry of using propaganda

THE US Government has accused its industrial partner in its fast breeder reactor development project at Clinch River, Tennessee, of producing pam-

phlets that give an "oversimplified" and "distorted" view of the dangers of nuclear power.

In a report published in Washington last week, the General Accounting Office said that the Breeder Reaction Corporation of Oak Ridge, Tennessee, which represents almost 700 companies involved in the fast breeder programme, had produced a series of thirteen pamphlets about the programme, at least four of which "clearly constitute propaganda and as such are questionable for dissemination to the public".

In particular, the GAO questioned the statement in one pamphlet that plutonium is "not a realistic threat when compared with other hazardous materials". It says that the pamphlet grossly understates the dangers, and does not inform readers that plutonium is extremely toxic, and in some circumstances carcinogenic. □

US ban use of prisoners in drug research

THE US Department of Health, Education and Welfare is planning to ban the use of prisoners for drug research not directly related to their health or well-being.

This suggestion follows last year's report of the Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, suggesting stringent conditions on such research to ensure that the participation of prisoners was voluntary.

In its response to comments on the Commission's report, published last week (January 6), the department says that the Commission found "a paucity of evidence" that research testing of drugs involving minimal risk on prisoners was necessary (see p. 197).

In issuing proposed regulations on research involving prisoners, the department has therefore suggested that the use of prisoners for biomedical and behavioural research studies other than those relating directly to the causes and conditions of their imprisonment, and their well-being in prison, should be banned.

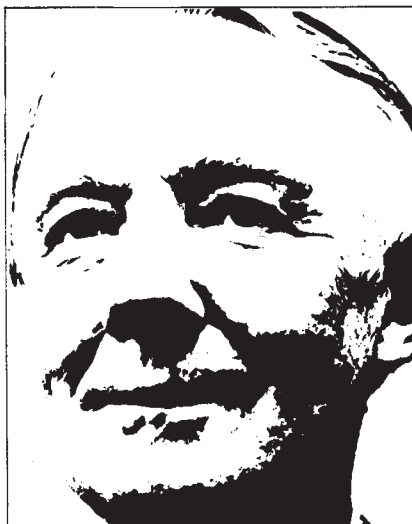
"The Department has concluded that the need to assure that research on human individuals is performed only on human subjects is performed only on individuals who have knowingly and voluntarily consented to participate far outweighs any need that has been shown for the use of prisoners in these studies" it says.

David Dickson

THE high rate of rickets in children of Asian origin in Britain is causing concern, and a demand that the government "does something about it". One suggestion is that various foods including the flour used to make chapatis, should be fortified with vitamin D. This proposal is being resisted by some scientists, because the present addition of calcium and vitamins to bread, and of vitamins to margarine, is now under attack. It is pointed out that there is little or no evidence that this costly and complicated scheme is having any beneficial effect on the nutritional standards of the population, and that more experimental evidence should be sought. It is probable that if every Asian child in Britain took a spoonful of codliver oil daily, rickets would disappear among this section of our population. Efforts to ensure that the vitamin went where it was needed would be more effective than giving everyone unnecessary and unwanted supplements.

Those who favour more mass fortification of food have the mistaken belief that as we all need small amounts of substances like vitamin D to preserve our health, then the more we consume of them the healthier we will become. This false opinion is perpetuated by many advertisements. I have before me one which tells me this preparation is rich in protein, iron and vitamins B1 and B2. It goes on to say that protein is essential for growth and bodily repairs, iron to nourish the blood, and the vitamins to aid digestion, convert food to energy and to maintain tissues such as nerves, muscles and the skin, and generally to promote "vitality". Seed

Enough is enough



KENNETH MELLANBY

merchants tell us that the more expensive varieties of vegetables give plants particularly full of protein, and with extra amounts of vitamin C and minerals. Once again they claim that these substances must be taken if we are to remain healthy.

All these statements about the need to eat protein and vitamins are of course true, and if we were living on deficient diets the advertised commodities would no doubt do us good. But when our diet contains the required minimum of these substances, and addition is generally useless and often harmful.

Unfortunately in Britain it is often the better educated who talk the most

nonsense about diet. They have learned some of the elements of nutrition and know all about deficiency diseases. They seldom realise that most of our over-fed adults have a surplus of protein and other essentials, and that most of the allegedly health-giving supplements would be best flushed down the drain.

Yet malnutrition, in various forms, is common. In all Western countries the most common and obvious form is gluttony with consequent obesity. Those who try to slim by eating less are subject to a barrage of advertisements for low-calorie foods containing large amounts of supplements. These are generally unnecessary, for the slimmer lives on a nutritious diet of (his own) human flesh. Then the appalling state of our teeth is a symptom of some dietary imbalance, partly but not entirely connected with our overconsumption of sugar. But on the whole most adults seem not to be short of protein and vitamins, so more of these are not "good for them". The only common deficiency would seem to be fibre, and there is evidence to suggest that added bran does some good, but most supplements seem to be useless or harmful.

The position with young children and the elderly is rather different. These groups have a limited food intake, and in Britain today up to half of their calories may come from sugar, in sweetmeats for children, in hot sweet tea for the old. This leaves little room for essential nutrients, so it may be necessary to add these in concentrated form. But it would be more sensible to cut down on the sugar, which, in excess, is always harmful.

correspondence

Soviet genetics

SIR,—Dr Italo Barrai in his letter (*Nature* 271, 8; 1978) commenting on my earlier article on the new controversy on human genetics in the USSR (*Nature* 268, 285; 1977) asks me two questions which I shall answer here.

Barrai questions my ability to describe the situation in genetics "... what can a gerontologist say about human genetics: in what position is he to pass value judgement?"

It is true that most of my research while in the USSR and here in UK had been related to problems of ageing. However, my book *The Rise and Fall of T. D. Lysenko*, published by Columbia University Press in 1969 is still the only book written and published by a Russian scientist about the history of genetic controversy in the Soviet Union. This book also described the history of medical genetics in the USSR from 1920 to 1967. My article in *Nature* was in some way a continuation of these accounts.

Barrai's second question asks that I prove that in 1976 N. V. Tsitsin was not yet appointed president of the Genetic Congress (which takes place in Moscow in August 1978). The proof comes in the decision about the Moscow Congress made at the previous Congress in Berkeley in 1973. At this Congress D. K. Beliaev, in his capacity of the President of the Soviet Genetic Society, made an invitation on behalf of the Government of the USSR, to hold the next Congress in Moscow. As soon as the invitation was accepted D. K. Beliaev started to make all necessary arrangements as acting President. N. V. Tsitsin's name as president of the Congress appeared only in 1977, and I believe that it surprised not only me, but also the International Genetic Federation. N. V. Tsitsin, now 80 years old, was never very popular among geneticists, mostly because the way in which he acquired prominence in the mid-1930s. One can read about this in the book by Prof. D. Joravsky *The Lysenko Affair* (Harvard University Press: Boston, 1970)—

"In 1932 Tsitsin moved to the West Siberian Experimental Station in Omsk, whose director, V. R. Berg, believed in the practicality of crossing wheat with couch grass. He had been

working on the problem himself.

"A year after Tsitsin's arrival in Omsk, Berg was arrested as a 'wrecker', and Tsitsin was made a hero by the mass media, even though (or because) he still complained that 'an enormous portion of specialists up to the present are extremely negatively inclined toward our work.' A commission of inquiry from the Commissariat of Agriculture, which was itself being purged of 'wreckers', decided that Tsitsin's work had enormous promise and merited great support. He was given space in *Pravda* to promise an annual hybrid of wheat and couch grass ready for production testing by the fall of 1935; a perennial would take a year longer. When 1935 came to an end without a hybrid ready for the testing service, Tsitsin received reassurance from the highest authority. Stalin told him, and allowed the awesome words to be inscribed on newsprint: 'Experiment more boldly. We will support you'. Tsitsin became the director of the Omsk station, which had been promoted into the Siberian Institute of Grain Culture. Specialists began to pay respectful attention to his work."

Everything in this description is correct. One can find details of the events in *Pravda* for 9 July 1934 and 30 December 1935, pp. 81–82. N. V. Tsitsin has still not made perennial wheat, but as a director of a big plant breeding research institute after the second world war he could be given credit for some achievements in wheat breeding. He has not played any serious role in Soviet genetics for the last five years; and his name was removed from the editorial board of the Soviet journal *Genetica* at the very beginning of 1973. If Soviet geneticists had had a free choice of who was to be president of the first International Congress on Genetics held in the USSR I doubt very much that Tsitsin would have got the job.

Dr Barrai also asked me to give a fuller justification of my negative assessment of N. P. Dubinin's current work. I am afraid that this needs more space than this letter permits. Dr Barrai, however, partly provided the answer himself. Trying to explain some false methods in N. P. Dubinin's publications Dr Barrai wrote "... it might well be that, as director, he signs work from his institute which might be

beyond the capacity of his technical judgement". Some of my colleagues in the USSR also thought that this could be the case. They thought, however, that this could equally be said about publications which were not failures. In any case the Moscow Congress will provide enough information about the situation in Soviet genetics today for those who do not restrict their interest to the official programme.

Yours faithfully,

ZHOES A. MEDVEDEV

National Institute for

Medical Research, London

NIH guideline ineffective

SIR,—The National Institute of Health *Guidelines for Recombinant DNA Research* recommended 2% aqueous Wescodyne, an iodophore that is used in many hospitals and laboratories as a disinfectant, as a decontaminant for biological safety cabinets and 5% for a spill outside a cabinet. A contact time of 10 to 15 minutes was given for the 2% solution and 20 minutes was considered adequate for the 5% concentration.

However I have conducted experiments which indicate:

- Aqueous Wescodyne (5%) is ineffective when used for 80 minutes against poliovirus in a test mixture containing 8.5% bovine serum albumin (a mixture equivalent in protein concentration to the higher range in serum).

- Wescodyne (10%) employed under the same conditions for 40 minutes is also ineffective.

- Wescodyne (10% v/v) in 50% ethanol (w/w) was effective and this mixture, originally recommended for hand washing, should be considered for use in biohazard situations, particularly for decontamination of work surfaces and biological safety cabinets.

These results are significant, for if a virucide cannot inactivate poliovirus one would be concerned about using the virucide against hepatitis B or SV40 viruses.

Yours faithfully,

ALFRED M. WALLBANK

The University of Manitoba,
Winnipeg, Canada

news and views

Pseudogene structure in 5S RNA genes

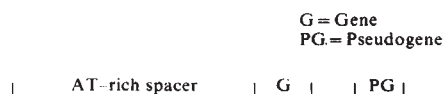
from Peter Ford

THE 5S RNA genes of *Xenopus laevis* have several obvious advantages as a system for studying specific gene regulation in higher eukaryotes. The 5S RNA itself is a small (120 nucleotides) readily purified molecule which is easy to identify by simple sequencing techniques. As a component of ribosomes it is found throughout the living world and has an essential though poorly understood function in protein synthesis. It is synthesised in its final form with no obligatory processing at either 5' or 3' terminus unlike most other RNA species, which means that correct termination and more important correct initiation of transcription may be easily assayed. In addition *Xenopus laevis* expresses different sets of 5S RNA genes in somatic cells and oocytes, a property which is phylogenetically widespread since it also occurs in other amphibians and teleost fish, and implies a general mechanism by which differential 5S gene expression is controlled. No one understands at present the functional significance, if any, of different 5S RNA sequence variants in oocytes and somatic cells, and for the purposes of understanding gene regulation this is not important.

D. Brown and his collaborators reported the purification of 5S DNA from *Xenopus laevis* a number of years ago (Brown, Wensink & Jordan *Proc. natn. Acad. Sci. U.S.A.* **68**, 3175; 1971). The genes are highly repeated (24,000 per haploid genome) and tandemly linked into several clusters (perhaps as many as 18) at the ends (telomeres) of chromosomes (Pardue, Brown & Birnstiel *Chromosoma* **42**, 191; 1973). As it turned out the purified 5S DNA prepared by Brown is of the oocyte type, the somatic type has not so far been isolated from *Xenopus laevis*, which means that the work done so far on purified 5S DNA relates only to the oocyte type sequences. The bulk of the

purified 5S DNA has a regular alternation of AT-rich with GC-rich sequence within each repeating unit which is about 700 base pairs long. Although different repeating units are very similar to each other they may differ in length and in sequence detail. Length heterogeneity has a quantal element resulting from a variable number of a 15 base pair mini repeat located in the AT-rich region but is complicated by presence or absence of other specific AT-rich sequences. The GC-rich region which contains a single 5S RNA gene is very constant in length (350 base pairs) but may vary in sequence detail.

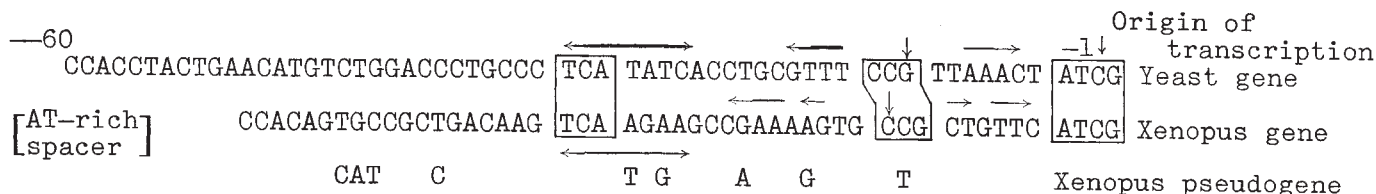
What has come as a surprise to many people is the recent demonstration by two independent sequencing techniques (Jacq, Miller & Brownlee *Cell* **12**, 109; 1977; Fedoroff & Brown *Cold Spring Harbor Symp. quant. Biol.* **42**, in the press) that the GC-rich region of 5S DNA also contains a pseudogene which is identical to the first 101 base pairs of the gene at all but 10 sites. The pseudogene completely lacks the last 19 base pairs of the gene. The pseudogene is detected in both total genomic 5S DNA as well as in purified individual repeat units cloned in bacterial plasmid vectors and can thus be assumed to occur in the majority if not all of the repeat units in oocyte type 5S DNA. A map of the repeat unit now looks like this



Attempts to show that the pseudogene is transcribed *in vivo* have not been successful suggesting that pseudogenes are not transcribed or that their transcription products are very short lived. Until positive evidence for pseudogene transcription is found it seems reasonable to assume that they are inactive. Now both the gene and

the pseudogene are preceded by GC-rich sequences 49 base pairs long which are identical in all but nine positions. By analogy with prokaryote systems the region preceding a gene is expected to contain promoter sites for RNA polymerase binding and perhaps regulatory sites for the control of transcription. Since all the information required for the correct initiation and faithful transcription of the 5S gene is present within one repeat unit as was shown by the successful and faithful transcription of cloned single repeat units after microinjection into *Xenopus* oocyte nuclei (Brown & Gurdon *Proc. natn. Acad. Sci. U.S.A.* **74**, 2064; 1977; Fedoroff & Brown *op. cit.*), it seems reasonable to conclude that the specific absence of pseudogene transcription is due to the absence of the information required for initiation of transcription in the pseudogene. This means that either the information for correct polymerase binding resides in the AT-rich region which comes before the GC-rich 'promoter' region of the gene or it has been specifically destroyed by some or all of the nine base changes which differentiate the 'promoter' region of the pseudogene.

The sequence for a 5S gene region from the budding yeast, *Saccharomyces cerevisiae*, has been determined recently (Valenzuela *et al.* *Nature* **267**, 641; 1977; Maxam *et al.* *Nature* **267**, 643; 1977) and it may be instructive to compare the 'promoter' region with that found in *Xenopus*. The rationale for this comparison lies with the observation that prokaryote promoters show AT-rich regions which by mutation analysis are known to be involved in polymerase binding. One site which allows initial recognition and entry of the polymerase is located about 30–35 nucleotide pairs upstream from the origin of transcription. A second AT-rich site, the tight binding site, is located 10 nucleotide pairs from the origin of transcription. There is some but not a great deal of sequence con-



ervation at these sites when different promoters are compared. Comparison of the yeast and *Xenopus* 'promoters' shows very little sequence similarity as expected, but there are two structural features in common and only future work will show if they are significant. A sequence with twofold rotational symmetry is centred 11 (yeast) and 12 (*Xenopus*) base pairs from the origin of transcription and contains a CCG sequence off centre. The second feature is the presence of an AT-rich region seven nucleotides long which has a trinucleotide in common and is centred 27 base pairs away from the origin of transcription. Both these sequences are mutated in the pseudogene 'promoter' consistent with the notion that they are important in the normal faithful origin of transcription.

The presence of an inactive pseudogene presents an evolutionary paradox. Since it is not transcribed it is prob-

ably non-functional although it may have some other unknown function for which it has been selected and preserved in evolution (Jacq *et al. op. cit.*), but the mutations which make it non-functional are present in every copy and must have arisen once in the original pseudogene. The alternative that the same mutations have occurred many times at the same sites in different pseudogenes is not only unattractive but a highly improbable set of events. As it now exists the pseudogene sequence would produce a partial 5S molecule which might compete with the normal product for ribosome binding but render such ribosomes inactive or inefficient in protein synthesis. There would be heavy selection against such a gene activity and mutations would rapidly accumulate to inactivate or eliminate the pseudogene. The evolutionary paradox is thus seen as a question how can a functionless

potentially deleterious sequence spread in a genome to the extent that the 5S pseudogene has spread as if selected for some particular purpose? Could it be as Fedoroff and Brown suggest that the events leading to the spread of the gene itself have also led to the spread of the pseudogene as an unwitting 'fellow traveller of an evolutionarily successful gene'?

Whatever the actual evolutionary history of the pseudogene it is clear that future work will establish the regions essential for polymerase binding and possibly for control elements in 5S DNA. Such experiments will make use of the *Xenopus* oocyte nucleus as a vehicle for testing mutated and mutilated cloned 5S DNA repeats. What of tissue specific control? It would be useful to have the somatic type 5S DNA cloned and purified for comparison but that is another story and must wait for another day. □

Rhizobium recognition

from John E. Beringer

ALL *Rhizobium* species have the capability to form nitrogen-fixing nodules on the roots of leguminous plants. However, not all legumes are nodulated and those that are usually interact only with a particular 'species' of *Rhizobium*. This host-range specificity has long been an intriguing problem but, until recently, little was known about its biochemical basis. An understanding of the mechanisms should not only enhance our knowledge of the symbiosis, but also indicate ways in which *Rhizobium* and plant species may be manipulated genetically to increase the range of nodulated plants.

It is thought that host range is determined before the bacteria penetrate the surface layers of the root and that it is based on the specific recognition of the 'correct' *Rhizobium* species by the plant in a manner analogous to antigen-antibody binding. What makes this an attractive model for the host-range interaction is the observation that plants produce compounds called lectins that are able to bind to 'antigens' and are known to play a part in the plant's defence mechanism against pathogenic microorganisms.

The lipopolysaccharides of Gram-negative bacteria are well known antigens, primarily because they include the O antigens of *Salmonella* species. The rhizobia are Gram-negative bacteria and share the same general pattern of surface structure as the salmonellae. Working outward from the cytoplasm this includes a mucopolysaccharide layer, then lipopolysaccharide, often a polysaccharide capsule and sometimes a further, less adherent, layer of structurally fairly simple exopolysaccharide. Because lipopolysaccharides are known antigens it might be expected that if there are host-specific antigens recognised by legumes they would reside in this layer of the cell wall.

There have been a number of reports in the literature which indicate that lectin binding by *Rhizobium* species may be involved in the host-symbiont recognition process. Dazzo and Hubbell (*Appl. Microbiol.* 30, 1017; 1975) have even produced a model for the recognition process based on the observation that a common antigen occurs on the surfaces of the roots of clover and on nodulating strains of *R. trifolii*. They suggested that the binding of multivalent lectins to these common antigens could be the method whereby the cor-

rect host and *Rhizobium* species were brought into close contact, allowing the interaction to proceed to a point where penetration could occur. Despite anomalies that have been reported in lectin-*Rhizobium* binding studies, it is now generally accepted that recognition is likely to involve lectin binding.

Having shown that lectin binding was important, an obvious step was to determine the nature of the bacterial antigen(s). Dazzo and Hubbell suggested that the antigenic determinant(s) of *R. trifolii* strains resided in the capsular polysaccharide and later Dazzo and Brill (*Appl. environ. Microbiol.* 33, 132; 1977) reported that this binding was mediated through 2-deoxyglucose-sensitive binding sites present on clover root hairs and in the capsular polysaccharide of nodulating *R. trifolii* strains. They also isolated the binding protein (lectin) of clover which bound to *R. trifolii* but not to two species which do not nodulate clover, *R. meliloti* and *R. japonicum*. This attractive piece of research served to indicate that at least the initial stages of host-symbiont recognition were simply based on the presence of a common receptor in the surface layers of the host and bacterium. However, the situation is not so clear because Wolpert and

Albersheim (*Biochem. biophys. Res. Commun.* **70**, 729; 1976) have reported that legume lectins interact with the lipopolysaccharide of their 'correct' *Rhizobium* species.

A classic way to examine the importance of cell wall components for antigenicity or phage attachment has been to examine mutants defective in their synthesis. This approach has been taken by Sanders, Carlson and Albersheim who report their findings with *R. leguminosarum* in this issue of *Nature* (page 240). By selecting bacteria after passing cultures through 1.2 μ m Millipore filters they obtained mutants at high frequency which did not produce exopolysaccharide. Chemical characterisation of the mutants indicated that there was no exopolysaccharide synthesised and that the capsule and lipopolysaccharide were unaltered. Five of these mutants, otherwise apparently indistinguishable from the parent, were tested for their ability to nodulate their correct host plant, the pea; all five mutants were non-nodulating. Five spontaneous revertants of one of these mutants, selected for the reappearance of exopolysaccharide production, all regained the ability to nodulate the pea. Thus it appears that the exopolysaccharide is essential for nodulation to occur, at least in this strain of *R. leguminosarum*. Whether or not it is involved in lectin binding has not yet been determined.

It now remains for these and other workers to clarify the role of capsular, lipo- and exopolysaccharides in determining the host range of *Rhizobium* species. Indeed it has yet to be shown how complex the host recognition process is. Perhaps lectin binding is only one of a complex series of recognition processes that must occur before a nitrogen fixing nodule can develop. □

Mining triggers earthquakes

from Peter J. Smith

It is almost universally accepted now that, given the right circumstances, earthquakes may be induced artificially by injecting fluids into the ground, by setting off underground nuclear explosions or by filling large reservoirs. Indeed, the idea is so commonplace that there may be some difficulty in believing that less than 7 years ago it was the subject of lively controversy, largely because the evidence in favour of man-made earthquakes, though strong, was then little more than cir-

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

Animal sounds: coloratura and basso profundo

from John Krebs

MANY species of birds and mammals use sounds as threat signals in contests over territory and other resources. Dogs growl, elephants roar, and great tits scold. The remarkable thing is that the threat sounds of most birds and mammals tend to be low-pitched, harsh (broad frequency spectrum) noises. In contrast non-aggressive signals, such as appeasement gestures, tend to be high pitched and contain pure tones. E. S. Morton (*Am. Nat.* **111**, 855; 1977) now suggests an explanation.

Threat signals in the animal world to some extent replace out-and-out fighting, presumably because it is less dangerous for an individual to settle a dispute in this way. But obviously the loser of the contest, which might be for example over a piece of food, does less well than the winner and so one might expect, anthropomorphically speaking, losers not to give up easily when faced with a threat signal unless they are sure that they would lose eventually. The inescapable conclusion is that at the beginning of a contest animals try to assess the fighting ability of their opponent without actually engaging in a scrap. What sort of cues therefore would give reliable information about an opponent's fighting ability? A totally arbitrary cue would be easily faked, but some body features are inevitably correlated with strength, an obvious example being body size.

The significance of low pitched threat sounds should now be apparent: on the whole, larger individuals are capable of producing lower pitched sounds. The pitch of a threat call depends on the tension, size and thickness of the vibrating membrane on the voice box, and at least in mammals, on the volume of the nasal and oral cavities which act as resonators. In other words, depth of voice gives a quick, rough indication of how big an opponent is, and so it is not surprising that low pitched sounds have, during evolution, become an effective way of threatening a rival. Harshness is often an inevitable by-product of low frequency sounds, since a vibrating membrane under low tension will tend to produce harmonically

unrelated tones.

Why should appeasement and non-aggressive sounds be high pitched? One possibility is Darwin's Principle of Antithesis—signals conveying opposite kinds of information tend to be very different to reduce confusion—but Morton also suggests that high pitched appeasement calls have, so to speak, parasitised the fact that animals respond in a non-aggressive way to the high pitched begging calls of their young.

Coloratura, according to my dictionary, refers to 'incidental trills introduced to make a song or other piece of music more agreeable.' The song of our common or garden wren (known across the Atlantic as the winter wren) has enough coloratura to make Rossini's *Una voce pocha* sound like a Gregorian Chant, but does this make it more agreeable to other wrens? D. Kroodsma (*Am. Nat.* **111**, 995; 1977), suggests that it does. He studied song recordings of the nine species of North American wren, which show a considerable range of complexity. Virtuosi such as the long-billed wren have over 100 different song types for each individual male, while the less ostentatious male Bewick's wren has between 9 and 20 song types. Kroodsma calculated for each of his nine species an index of song virtuosity, including information about the number of song types and the complexity of each type (for example our own wren has few song types but each one is very long and complex, so this species scores high on overall virtuosity.)

One conclusion of this analysis is that wren species with the most complicated songs are those species in which males are most polygynous. In polygynous species, the males compete intensely for females to add to their harems, and as the females have a choice of mate, they exert powerful sexual selection on males. Any male trait which increases his attractiveness to females will be favoured, and it seems that coloratura singing in wrens is just such a trait. It is an auditory peacock's tail.

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cumstantial. What was lacking in most cases was a detailed record of seismic conditions for the period before the suspect events had become quite ob-

vious even to the least sensitive member of the population. In short, 'before and after' seismic comparisons were generally not available—a data gap

which led inevitably to a credibility gap.

Thanks largely to the controlled experiments at the Rangely oil field in Colorado (see for example Raleigh *et al. Science*, **191**, 1230; 1976) and the wider recognition of the role played by fluids in seismic processes generally, the reality of man-made earthquakes is no longer in doubt. Nevertheless, well-documented examples of the onset of such events are still quite rare, which makes the observations in western New York state by Fletcher and Sykes (*J. geophys. Res.* **82**, 3767; 1977) of more than routine interest. They also exemplify astonishing good luck. Fletcher and Sykes set up their seismometer array in 1970 in anticipation of the injection of fluids into a deep well at a steel plant just south of Buffalo. The planned injection never actually took place, but seismic activity increased anyway. What Fletcher and Sykes were unaware of at the time was that a hydraulic salt-mining operation was about to begin nearby at Dale, New York.

As it happens, the Attica-Dale area is already the most seismically active region of western New York state, but only in that there is a history of isolated, moderately large earthquakes. There have apparently been no swarms of smaller events in the recent past; and certainly none was recorded by Fletcher and Sykes from June 1970 to early August 1971, a period during which there was an average of only one seismic event a month. Unfortunately, the recording equipment was out of action for much of August, September and October 1971; but when normal service was resumed at the end of October, Fletcher and Sykes found they were observing microearthquakes at rates of up to 80 a day. The contrast with the year to August 1971 was startling.

The foci of these events were found to lie in the vicinity of a high-pressure injection well used in the hydraulic mining of salt. If Fletcher and Sykes had known of this operation in advance they would probably have taken greater care to have their recording apparatus in working order at the crucial time in early August 1971 when the well pressure was raised to its maximum of more than 60 bars. As it was, the onset of pressure-induced earthquakes went largely unrecorded. On the other hand, there is little doubt that the high activity observed from October onwards was the result of well fluid pressure, for when that pressure was allowed to fall suddenly to below 10 bars in mid-November the microearthquakes ceased equally spectacularly.

An important clue to the direct origin of the earthquakes comes from their distribution, for their foci lie not

only close to the injection well but also in an elongated zone along the Clarendon-Linden fault which passes within 50 m of the well bottom. The

obvious interpretation is that increase in pore pressure resulting from hydraulic mining triggered seismic activity in the Clarendon-Linden fault zone. □

Functions of the septo-hippocampal system

from A. H. Black

The Ciba Symposium on the Functions of the Septo-Hippocampal System was held in London on October 17–20, 1977. The proceedings will be published as Ciba Foundation Symposium 58 (Elsevier/Excerpta Medica/North Holland, Amsterdam and New York, 1978). The symposium was organised by Dr J. A. Gray and chaired by Dr L. Weiskrantz.

ALTHOUGH considerable attention was focused on the anatomy and physiology of the septo-hippocampal system, the symposium was primarily concerned with behavioural functions. Let me first point to a difference between the septum and hippocampus. Both H. Ursin (University of Bergen) and S. P. Grossman (University of Chicago), who described research into the effects of septal damage, argued that the septum was a complex structure involved in a number of different, perhaps unrelated, behavioural control circuits, and, therefore, that one could not ascribe a single or even a few functions to it. On the other hand, the discussion of the hippocampus (and those areas of the septum to which it was intimately connected) suggested that it might carry out a small number of related functions. I shall focus on the discussion of those functions.

There was little support for the hypothesis that the primary function of the hippocampus is to inhibit behaviour. This hypothesis dominated the non-human literature in the late 1960s and early 1970s. Its relatively sudden demise (perhaps only temporary) is all the more striking. Three other possible functions of the hippocampus received considerable attention. The first was the plasticity of the hippocampus and its possible role in learning. P. Andersen (University of Oslo) presented some elegant data on

the synaptic potentiation that follows repetitive stimulation in isolated transverse slices of hippocampal tissue. After short periods of tetanic stimulation of isolated input fibres to CA1 cells there was a long lasting (from 30 to 75 min) increase in the extracellular excitatory postsynaptic potential recorded from the apical dendrites. Also, single units showed a corresponding increase in their probability of firing. These changes were seen without any change in the size of the afferent fibre volley. These results indicated that the potentiation could be attributed to changes at the synapse—either to an increased amount of transmitter or to a changed postsynaptic resistance locally in the dendritic tree. Additional data on the plasticity of the hippocampus were provided by G. Lynch (University of California) from an experiment in the freely moving unanaesthetised rat. The rats were first trained to press a lever when a tone was sounded. During the course of such training an evoked potential recorded from the outer molecular layer of the dentate gyrus gradually developed to the tone. After training to a single tone had been completed, the rats were trained to discriminate between two tones. The evoked potential was now seen to both tones, and the tones elicited discharge of the granule cells even though they had not done so before. At first, fairly long bursts of single unit activity were observed to both tones. But, as time passed, long bursts of firing to the reinforced tone continued while the burst of firing to the non-reinforced tone decreased in duration. Lynch suggested that these results are consistent with the view that the tones produce an input to the hippocampus by way of the perforant path; the effects of the input increase gradually during the early stages of learning—perhaps by the mechanism described by Andersen above, and earlier by Bliss and Lomo. Whether this input results in cellular discharge depends, however,

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MANY plants, particularly aquatic ones, experience periodic or even permanent waterlogging in their environment. The selective advantages conferred by morphological adaptation to such conditions is evidenced by the widespread occurrence of lacunae and large intercellular spaces within aquatic species. It has been demonstrated that oxygen penetrates along such interconnecting channels, reaching the root or rhizome extremities. For example, Coult and Vallance (*J. exp. Bot.* **9**, 384; 1958) showed that oxygen moved the full length of a 45-cm rhizome of *Menyanthes trifoliata* when it was suspended in an oxygen-free atmosphere but with the leafy shoot exposed to air.

Individual cells, nevertheless, may find themselves in conditions of low oxygen tension. Under such conditions, metabolic adaptations are essential, particularly the avoidance of ethanol accumulation as a result of anaerobic glycolysis. McManmon and Crawford (*New Phytol.* **70**, 299; 1971) examined a range of flood-tolerant and flood-intolerant species under waterlogged conditions and they found that some species, such as *Pisum sativum* and *Vicia faba* which are flood-intolerant, showed greatly increased levels of alcohol dehydrogenase (ARH, involved in acetaldehyde to ethanol conversion) under flood conditions and also high activity of 'malic' enzyme which converts malate to pyruvate (which can then be converted to acetaldehyde and hence to ethanol). Flood-tolerant species, such as *Phalaris arundinacea* and *Glyceria maxima* were found to decrease in their ADH activities as a response to flooding and showed no activity of 'malic' enzyme.

McManmon and Crawford proposed that flood-tolerant species lack malic

Adaptations to waterlogged environments

from Peter D. Moore

enzyme so that a diversion of glycolysis to malate production does not result in further malate decarboxylation to rejoin the path of anaerobic glycolysis and ethanol production, but ends in malate accumulation. Malate build-up seems to be harmless in these cells. In the flood-intolerant species any malate produced is decarboxylated by 'malic' enzyme, ADH is induced and ethanol accumulates to the detriment of the cell membranes.

Crawford and Bains (*New Phytol.* **79**, 519; 1977) have recently demonstrated that the build-up of ethanol in the roots of forest trees on waterlogging varies from one species to another. Thus, in the Sitka spruce (*Picea sitchensis*) which is intolerant of flooding, ethanol concentration had increased to 12 times its original level (to 5.6 $\mu\text{mol per g}$ fresh weight) after 24 h in anaerobic conditions, whereas in *Pinus contorta*, a flood-tolerant species, ethanol accumulation increased only three times its base level (0.7 $\mu\text{mol per g}$ fresh weight). Once again, tolerance to flooding is associated with a control of ethanol accumulation.

Crawford has now (*New Phytol.* **79**, 511; 1977) examined the response of certain seeds to anoxia, for some mire plants can survive for considerable periods as seeds in anaerobic environments. For example the seeds of the rush *Juncus effusus* are reputed to germinate after 7 years submergence.

Sensitivity to prolonged soaking was found to vary with species. Rice seed viability was unaffected by a 6 h soaking, broad bean was reduced to 40% and pea was rendered completely non-viable after such periods.

In the species intolerant of soaking, such as pea and maize, ethanol was always found to be the major product of glycolysis under anaerobic conditions. Malic acid did not accumulate, but lactate was high in the early stages of glycolysis in broad bean and rice, the species tolerant of soaking. Ethanol production, however, exceeded lactate in even these species after 24 h soaking. The overall rate of glycolysis under anaerobic conditions was greater in the sensitive species, hence ethanol accumulated faster in these species.

Thus it would seem that the tolerance of seeds for prolonged immersion in water depends not upon any metabolic 'switch' as is the case for many roots, but upon the capacity to reduce overall metabolic rate. Crawford has further shown that for five out of six Gramineae species which he kept for 6 weeks as imbibed seeds under anaerobic conditions, there was a reduction in viability with increasing temperature. This would again suggest that increased metabolic activity leads to seed death, probably by ethanol accumulation and the associated membrane changes.

Crawford thus maintains that, although the precise metabolic techniques may vary between species and even between organs, the essential problem involved in the toleration of anaerobic conditions is the control and limitation of ethanol accumulation. □

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on input through the second major afferent pathway to the hippocampus from the septum. Just what conditions lead to these plastic modulatory effects on the second input are not yet clear, however. Further research on these preparations should lead in the near future to a better understanding of plasticity in the hippocampus during learning.

The second function of the hippocampus that received considerable attention was its possible role in the detection of changed environmental circumstances, and in the behavioural adjustments that immediately follow the detection of such changes. While many participants agreed that the hippocampus is involved in the detection of change, they differed about the nature of the particular events whose change was detected. For example,

O. S. Vinogradova (USSR Academy of Sciences Biological Centre, Puschino-Oka) suggested that the hippocampus is involved in the detection of novelty (that is, any change in stimulus input), J. A. Gray (University of Oxford) that the hippocampus is involved in detecting changes in reinforcement schedule—in particular, from reward to non-reward, and J. O'Keefe (University College, London), on the basis of a theory that he and L. Nadel have proposed, suggested that the hippocampus is involved in setting up spatial maps of the environment and in detecting mismatches between the environment and the map when the former is changed. Given the extent of the agreement that the hippocampus plays a part in the detection of stimulus changes, one hopes that agreement as to the nature of these

changes is not too far off.

A third proposal was the hypothesis that the hippocampus is involved in certain types of spatial information processing. O'Keefe and A. H. Black (McMaster University) presented both single cell and lesion data in support of the O'Keefe and Nadel spatial theory of hippocampal function mentioned above. For example, certain hippocampal cells seem to fire only when a rat is in a particular location in a given environment, and their firing is independent of the rat's behaviour. D. Olton (Johns Hopkins University) also presented lesion data which were consistent with the view that the hippocampus is involved in spatial memory. He trained rats on an apparatus which consisted of a central circular platform from which eight arms radiated. Food pellets were placed

at the end of each arm. Normal rats very quickly learned to retrieve all eight pellets without re-entering an arm in which they had already found food. Rats with damage to the hippocampal formation failed to do this. But their performance, according to data provided by G. Winocur (Trent University, Ontario), improved if the floor of each of the eight arms was identified by a salient cue. It would seem that the lesioned rats could not employ spatial cues provided by the environment outside the maze to identify the location of each arm but could use specific non-spatial cues on the floor of each arm to do so. Although it is still too early to evaluate the spatial hypothesis, it seems to have aroused considerable interest—and controversy.

A final point concerns the problem of species differences. There are marked differences among species in a variety of aspects of hippocampal function, most of which are as yet unexplained. For example, the environmental events which elicited hippocampal single unit activity in the rabbit seem to differ markedly from those in the rat. Also, as C. Vanderwolf (University of Western Ontario) noted, more hippocampal theta electroencephalographic activity unrelated to movement occurs in the rabbit and cat than in the rat and dog. Perhaps the most important species differences are those between man and the other animals. A striking feature of the effects of hippocampal damage in man is an apparent memory deficit; experiments on the other animals show no memory deficit in most situations. Is this a real species difference or is it a procedural artefact? Winocur suggested that it was the latter. According to his view, after hippocampal damage subjects cannot use contextual cues properly to retrieve information in memory. The difference between man and the other animals arises because human subjects are usually tested on a series of learning tasks which interfere with each other unless contextual cues are used to isolate the tasks, while animals are tested on single tasks for which no interference occurs. Winocur argued that if the same type of task had been used in both cases, the deficit would have been similar. L. Weiskrantz (University of Oxford) suggested, however, that the difference may be real. He proposed that the hippocampal changes result in a dissociation between levels of processing. For example in a simple associational task, the human subject cannot talk about what he has learned to associate even though the associational learning is intact. Presumably, animals show no memory deficit because they lack the different levels and communication between levels of processing does not

occur. Which, if either, of these conjectures is correct remains to be seen. In any case, the concern with the attempt to integrate the human and non-human literature is a most important aspect of the problem of species differences and it is gratifying to see so much concern with it at present. □

Sociology of astronomical innovation

from David W. Hughes

SOCIOLOGY is a subject usually omitted from the degree courses of science and engineering students. They are apparently missing a lot; the fascination of the discipline is ably illustrated by a recent paper in the *Quarterly Journal of the Royal Astronomical Society* (18, 326; 1977) by David Edge of the Science Studies Unit, University of Edinburgh.

Edge applies himself to the problems of innovation—the processes of scientific discovery, the invention and introduction of new theories—and he discusses the ways in which astronomy compares and contrasts with other sciences.

Thomas S. Kuhn (*Sociology of Science*, Penguin Books, 1972) has asserted that the general run of scientific activity mainly consists of attempts to force nature into the conceptual boxes supplied by professional education. The scientist is busily accumulating systematically related knowledge within established areas, he is the objective, open-minded searcher after truth, the explorer of nature, the person who rejects prejudice at the threshold of his laboratory. During his education the scientist acquires a set of standards and a collection of mental tools and manual techniques which he can subsequently deploy in his own creative work. He is a puzzle-solver rather like a chess player. Education simply provides him with the rules of the game being played at the moment, initiating him into a pre-established problem-solving tradition without inviting him or equipping him to evaluate that tradition.

Much research is fettered by preconceived convictions and seems to demand a deep commitment to the *status quo*. A belief in the probable success of a venture is obviously an

important motivating factor. Very few scientists would design and build elaborate special-purpose apparatus or would spend months trying to solve a particular differential equation without a quite firm guarantee that their efforts had a high probability of success and would lead to publications and increased status amongst their scientific peer group. The mature scientist can anticipate with considerable precision the sort of result his research should lead to. Knowing what to expect he is then in the ideal position to recognise the anomalous event.

Because gremlins and temporary faults crop up in the large majority of research projects a new discovery often only results when the failure stubbornly refuses to go away. The resolutions of these failures often bring accepted theories and experimental techniques into question thus making the scientific discoverer a controversial figure, a perpetrator of revolutionary thoughts and a swimmer against the normal tides of scientific conservatism.

Very few scientific innovations are made simply by extrapolating from the known. Most are accidental. However, discoveries are not just lying around ready to be tripped over by the aimless wanderer. A high degree of scientific skill is required to discriminate between the key to a new discovery and mere system (or brain) failure.

David Edge has paid special attention to innovations in modern astronomy. His first point is that the large majority of the innovations were not made by astronomers at all but by people who are marginal to the profession. For example, the pioneer British radioastronomers were all physicists, most of them working on applied research. Interestingly the astronomical community at the time tended to ignore the discoveries, and it was many years before a single astronomer changed his experimental strategy and adopted the new radio technique.

The mobility of physicists also seems to be a crucial factor in astronomical innovation. Their experimental competence enabled them to produce results in, for example, the radio, radar, infrared and X-ray fields for an astronomical audience who were not so equipped. Also physicists were not fettered by the stereotype view of the stars and the Universe inculcated into the minds of the professional astronomers. Again innovators seem to thrive if they are one of a large group of scientists (a university department for example) which can provide support for the push into the unknown.

Most astronomical discoveries have been serendipitous, a chance happening in the pursuit of something else. Far too infrequently did discovery come from

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the logical progression of hypotheses, the prediction of a possible new kind of observation, the design, construction and the use of an apparatus to search for the predicted phenomenon and the eventual success, the discovery. In recent times only the discovery of the 21-cm hydrogen line fits this idealistic progression.

A more controversial point in Edge's paper concerns the reception of innovation by scientists. In some disciplines it seems that innovators are treated with apathy and hostility, forced to organise their own societies and journals, mainly because it was thought that the innovation tended to devalue the importance of the hard won skills and competence of established practitioners. Here astronomy seems to be anomalous, most innovators being happily supported and encouraged. Maybe this is because the astronomical innovators are not competing for scarce research resources against members of the established discipline. The pioneers of radioastronomy, for example, were supported by physics and engineering groups.

The final point concerns the strategy of a scientist when faced with a range

of options for future research. To undertake an obvious experiment is to risk outright competition with other workers and possible loss of priority. Attempting a risky or speculative experiment, however, courts failure and also lessens enormously the audience which can appreciate its significance. Most scientists aim for the middle course, trying to prevent duplication and direct competition and trying to ensure success. Innovation, however, opens up a new era into which researchers rush, thus leading to a brief spell of outright competition and its concomitant secrecy.

Edge's paper helps lay to rest the scientists' impression of sociologists as a happy band rambling through science and occasionally stumbling on the obvious with shrieks of amazement. Does an understanding of sociology help the individual scientist? David Edge parries this question by answering that "if you want the (scientific) game to go better, then it is surely necessary to be clear about what kind of game it is". So we may conclude that the sociology of this subject is important, is useful, and should be more widely available to the scientific student. □

leukaemic cells were lightly fixed with glutaraldehyde; these were then used to immunise mice, and subsequently to assay antibody binding activity. When the spleens from immune mice were chopped into fragments and placed in microwell cultures, the antibody produced from each fragment could be assayed and from other studies it has been shown that under appropriate conditions each culture well contained one or fewer antibody-producing clones. The second key point of technique was the use of an iodinated rabbit anti-mouse immunoglobulin probe to detect antibody binding to the glutaraldehyde-fixed target cells. Under optimal conditions of spleen fragments culture and microassays for antibody binding, it was possible to obtain sufficient antibody from a single culture well to perform 2,000 assays. In practical terms it means that this technique can provide tumour-specific antibody (by selecting the appropriate culture well) which is tailored to suit the requirements of an individual patient, and in sufficient amounts to monitor accurately the numbers of leukaemic tumour cells during the course of therapy. In short, this technique could provide a major step forward in the management of tumours where chemotherapy is effective but must be closely regulated according to the tumour load.

Serological analysis of human melanoma antigens using autologous sera and cultured melanoma cells from tumour-bearing patients has revealed a variety of antigenic specificities (A. Deleo, Sloan-Kettering). A similar conclusion was reached by S. Ferrone (Scripps Clinic) who tested sera from melanoma patients against a panel of five cultured melanoma cell lines. In both studies there was no evidence of a common tumour-specific antigen as defined by patient sera. However, R. Reisfeld (Scripps Clinic) has shown that a single glycoprotein linked to β_2 -microglobulin can be shown to carry melanoma-specific antigens as defined by a heterologous rabbit antiserum. Putting the available data together, the tentative suggestion emerged that while one, or a few, molecules may carry several antigenic determinants which are tumour specific, there is substantial variation between individuals in the particular constellation of antigens present. In fact one wonders if the HLA system, in which three closely related molecular species carry a cluster of antigenic determinants which vary from one individual to the next, may be an important clue to introducing some order into this field. However, in the absence of genetics, improved chemistry is required to separate these related molecules, and improved serology to elucidate the relationships. □

Analysing tumour antigens

from Rod Langman

The Armand Hammer Cancer Workshop on Tumour Antigens was held at The Salk Institute, San Diego, on 26-29 September, 1977.

A SMALL group representing a wide range of interests met to discuss various aspects of tumour antigens. Classical serological techniques have provided the backbone of current knowledge, but as finer distinctions become necessary the limits of conventional technology in raising and absorbing antisera are being approached. This point was particularly well made during the discussion of chemically-induced tumour antigens. For example, A. Deleo (Sloan-Kettering) used a methyl cholanthrene-induced tumour of Balb/c mice (Meth-A), which had no detectable C-type virus antigen expression, to immunise syngeneic and semi-syngeneic mice. He found that what should be tumour-specific antisera had high levels of nonspecific activity as assayed on a wide range of normal and tumour tissues. These antisera were apparently loaded with antibody against C-type

viral antigens which was produced when, under the stress of immunisation, endogenous C-type virus was induced. Despite extensive absorption the residual tumour-specific antibody still showed unexplained cross reactions that could not be further analysed. H. Festenstein (London Hospital) reported that the methyl cholanthrene-induced P815 tumour has apparent alterations in the H-2 alloantigens, as detected by standard reference antisera. From this and a number of similar observations it was proposed that tumorigenesis may involve modulated expression of H-2 alloantigens. While this interpretation was not widely accepted at the workshop, no competing explanation could be offered. Without better defined monospecific antisera it seems that antigens on chemically-induced tumours will remain elusive.

Although an obvious approach to preparing monospecific antibody, the Milstein-Galfré myeloma hybridisation technique has not yet been applied to tumour antigens. However, R. Levy (Stanford University) did introduce a variant of this idea when he described his adaptation of the Klinman spleen-fragment method for preparing antibody to human leukaemic cells. First, large numbers of the patient's leu-

Isoscalar breathing mode states identified in ^{144}Sm and ^{208}Pb

from P. E. Hodgson

OVER the past few years many collective multipole excited states of nuclei have been found, mainly by inelastic scattering measurements. The energy spectra of the inelastically-scattered particles show separate peaks at the higher energies due to the excitation of the low-lying states and a broad peak at lower energy due to the excitation of multipole resonances. The multipolarity of the resonance can usually be found by studying the angular distribution of the resonance peak, and comparing it with the distribution calculated from a model of the resonant state with various assumed multipolarities. In this way much has been learnt about the giant quadrupole resonance, but so far it has proved difficult to identify the monopole or breathing-mode state. This is called the breathing mode because it corresponds to the nucleus changing size but not shape, and this shows that its energy is closely related to the nuclear incompressibility.

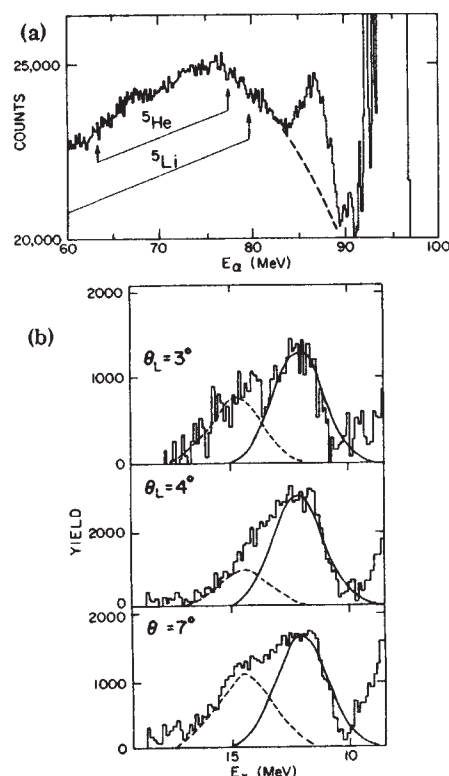


Fig. 1 Energy spectra of the α particles inelastically scattered from ^{208}Pb (a) and ^{144}Sm (b) showing the analysis of the giant resonances into two peaks.

In a recent experiment, Youngblood and colleagues of Texas A&M University have found that inelastic α -particle scattering at forward angles provides clear evidence for the breathing-mode excitation in ^{144}Sm and ^{208}Pb (*Phys. Rev. Lett.* **39**, 1188; 1977). In their earlier work with 97 and 115 MeV α particles they had found that the giant resonance peak has two components, but both had the same angular distribution and were assigned to the giant quadrupole resonance.

The new experiments were made with 98 MeV α particles, and measurements were made at much smaller angles than before, between 3° and 8° to the incident beam. Some of their results are shown in Fig. 1, together with the analysis of the giant resonance peaks into two components. The angular distributions of the two peaks are shown in Fig. 2, and it is clear that they are very different, especially around 4° . The multipolarities of the resonances were found by making calculations of the angular distributions assuming that the states are monopole, isovector dipole, quadrupole and hexadecapole, corresponding to $L = 0, 1, 2$ and 4 respectively. It is clear in each case that the data for the component at the lower energy is well fitted by the quadrupole curve and for the component at the higher energy by the monopole curve in each case. In particular, the predicted signature of the monopole state, a sharp minimum around 4° , is very clear in the data for the excitation to the higher energy for both nuclei, while it is absent in the data for the lower energy excitation.

The giant multipole excitations should satisfy sum rules, which means that the integrated strength of all their components should add to a value that can be calculated from the theory of the excitations. In the present experiments, it is found that all four resonances satisfy the energy-weighted sum rule to within the uncertainties of the analysis. This is a valuable confirmation of the correctness of the assignments.

The results for the breathing-mode excitation energies are in good agreement with previous experimental work, and with the recent theoretical estimates. According to the liquid drop model, the monopole excitation energy is related to the nuclear incompressibility K by

$$E_0 = \pi/3R(\hbar^2 K/m)^{1/2}$$

where R is the nuclear radius and m the nucleon mass. The excitation energies 13.7 MeV for ^{208}Pb and

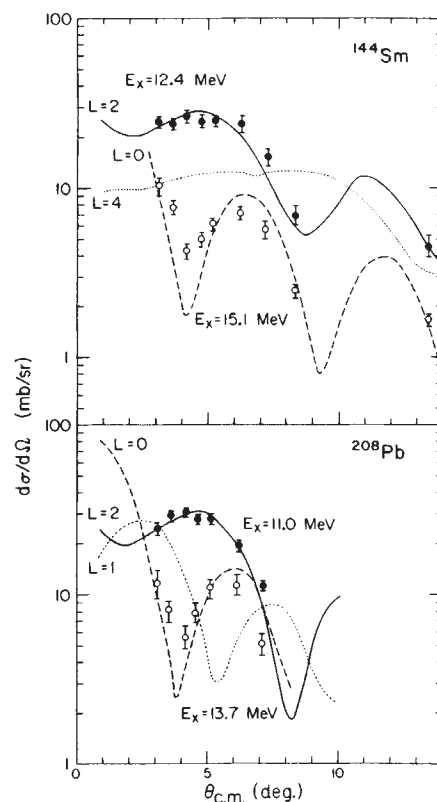


Fig. 2 Angular distributions of the two components of the giant resonance peaks in ^{144}Sm and ^{208}Pb compared with distorted wave calculations assuming various values of the multipolarity L .

15.1 MeV for ^{144}Sm give values of 208 and 197 MeV for K respectively, which are consistent with other theoretical estimates. □



A hundred years ago

MR. STANLEY will probably arrive in England this week. He has been received with enthusiasm at Rome, Marseilles, and Paris. The Chamber of Commerce and the Geographical Society of Marseilles presented Mr. Stanley with medals. No doubt our own Geographical Society will take the lead in the warm reception which will certainly be accorded in this country to one of the foremost of explorers.

THE wolves in Eastern France have become unusually bold during this winter, and reports are constantly received of their depredations in various parts of the country. In one instance a letter-carrier was driven back by them from his regular route.

From *Nature* 17, 17 January, 232; 1878.

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review article

Why has Regge pole theory survived?

Elliot Leader*

In spite of a very mixed record of success and failure, Regge theory, in which the notion of complex angular momentum is introduced into quantum mechanics still lives on. What has dissuaded physicists from discarding it, and what is our present assessment of the theory?

PHILOSOPHERS of science, who ponder the survival and demise of scientific theories, would do well to test their ideas against the progress of Regge Pole Theory since its inception in 1959.

Tullio Regge's great imaginative leap, the introduction of the notion of a complex angular momentum in non-relativistic quantum mechanics¹, might have ended in oblivion, weighed down by its overpowering mathematical sophistry and rigour, had not S. Mandelstam, seizing upon its crucial element and casting off the enveloping mathematical shroud, demonstrated a direct and striking consequence in the behaviour of high-energy elementary particle collision processes (Mandelstam did not publish this work, but his role is acknowledged in many papers).

Thereupon Regge theory really took fire and blazed through the ranks. In no time at all 'Regge pole' (pole in the mathematical sense) had become a household expression. Indeed there is the story of a party at which the charming wife of an American physicist, on being introduced to 'Regge', exclaimed: "Ah Mr Pole! I'm so pleased to meet you at last."

What is it that Regge did? What were the consequences? Where does the matter now stand?

Much of elementary particle physics is concerned with the scattering amplitude F , a function which controls the probable outcome of a collision between two particles. In the simplest case F depends only upon the energy of the collision E and upon the angle θ through which one of the particles is deflected. The behaviour of $F(E, \theta)$ reflects, albeit in a complicated way, the nature of the fundamental dynamical forces acting between the particles; hence its importance.

In ordinary quantum mechanics, where the forces are known, $F(E, \theta)$ is deduced by solving the Schrödinger Equation. Traditionally it was always simplest not to aim for $F(E, \theta)$ itself, but rather to study the collision at a fixed value of the angular momentum l . The forbidding Schrödinger Equation breaks up into a set of much simpler equations, one for each value of l ; l of course, being restricted to positive integer multiples of Planck's constant, as it must be in quantum mechanics. Thereby one obtains a set of amplitudes $f_l(E)$, $l = 0, 1, 2, \dots$, from which it is possible to construct $F(E, \theta)$ itself.

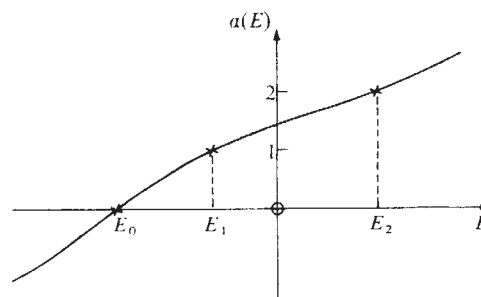
It has long been known that there are certain advantages in adopting the fiction that the energy E can be a complex number, that is, have both real and imaginary parts, and in considering each $f_l(E)$ as a function of the complex variable E (refs 2,3). The remarkable consequence of this bizarre strategy is the following. Suppose that the two particles whose collision is under study are capable of binding together to form a stable bound state, say with angular momentum L and (negative) energy E_B . Then the L th one of the above functions, that is, $f_L(E)$ will be found to have a 'pole' at the negative value $E = E_B$, that is, a simple infinity of the form $1/(E - E_B)$. The continuation of the energy E from its real positive

physical values to complex and negative values has unified the consideration of scattering processes and the existence of bound states.

With hindsight it is obvious that the next, even more bizarre, step ought to have been to try to allow the angular momentum l to take on non-integer, and indeed, complex values. But between the generalisation of the smoothly varying energy E to complex values and a like extension of the variable l , which physically took on only the values $0, 1, 2, \dots$, lay a great psychological barrier. Indeed 33 years were to pass before Regge's brilliant papers laid down the foundation of the theory of complex angular momentum.

Of course, Regge did not complexify for the sake of complexification. It is probable that he saw the introduction of complex l principally as an aid toward proving certain mathematical properties of the scattering amplitude that he had been trying to establish. The harvest was richer than expected.

First there emerged a new way of classifying stable bound states and resonances (quasi-stable states) into families. Instead of focussing separately on a bound state with a given angular momentum, say $l = 0$ with energy E_0 , and then upon one with angular momentum $l = 1$ with energy E_1 , and so on, one now focussed on a single object, the 'trajectory function' $\alpha(E)$ with the property that if a particular value of E should cause $\alpha(E)$ to equal a positive integer, say L , then a bound state would exist at that energy E and with angular momentum $l = L$. Thus in the above example the bound states would correspond to finding $\alpha(E_0) = 0$, $\alpha(E_1) = 1$, and so on. The curve $\alpha(E)$ against E signals the existence of bound states or resonances if and when it passes through integer values.



The bound states at energies E_0 , E_1 , and the resonance at E_2 (positive energy) are united into a new kind of family characterised by their trajectory function.

Second, it was found that just as $f_l(E)$ has a pole, that is, is infinite at the energy E for which a bound state of angular momentum $l = L$ exists, so now $f(l, E)$ has a pole, a 'Regge pole' whenever E is such that $\alpha(E)$ equals the value of l under consideration. The Regge pole coincides with the physical bound

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states or resonances when this value happens to be an integer.

Third, it was established that in the quite unphysical region of very large $\cos \theta$ (that is, $\cos \theta \gg 1$) the scattering amplitude is proportional to $(\cos \theta)^{\alpha(E)}$. In some mysterious way the Regge trajectory function $\alpha(E)$, so intimately linked to the properties of the bound states, is also responsible for controlling the growth of the scattering amplitude in the weird region of large $\cos \theta$.

This, in the broadest of terms, was the achievement of Regge. Whatever he proved is unquestionably true and will remain so as long as quantum mechanics holds sway. Indeed Regge theory has been included in good undergraduate text books for more than a decade.

Beautiful though this all is, it is very heavy going mathematically and might have remained undigested for a long time had not Mandelstam emphasised that in the real world of relativistic physics the quite meaningless limit of large $\cos \theta$ in the reaction $A + B \rightarrow A + B$ is closely related to the physically sensible, and exceedingly interesting, limit of high energy in the related reaction $A + \bar{A} \rightarrow B + \bar{B}$ involving the antiparticles $\bar{A}$, $\bar{B}$ of A and B .

The full story is technical and difficult, but what emerges is remarkably simple and quite dramatic: the behaviour of the scattering process $A + B \rightarrow A + B$ at very high energies E is proportional to the energy raised to the power $\alpha(t)$ that is, $E^{\alpha(t)}$, where α is the Regge trajectory connected with the bound states and resonances of the $A\bar{A}$ or $B\bar{B}$ systems. (t is the square of the 'momentum transfer' in the scattering—roughly $(E\theta)^2$).

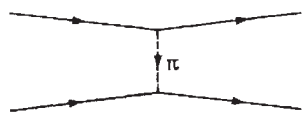
Two points are noteworthy: that there is such a close link between high energy behaviour and the properties of the bound states and resonances, and that the behaviour thus predicted at high energy, in which the power to which E is raised depends upon t (and therefore upon θ), is quite in contrast with what had been expected.

Like theologians of old taunted by the threat of a heliocentric solar system, the experimentalist rushed forth to destroy the theory. Amazingly, the first results from CERN in 1961 seemed to support the theory. Proton-proton scattering was changing with energy as predicted. (The diffraction peak was shrinking.) Enraged, the experimentalists tried harder. (There was the famous slide, shown at a conference in 1963, in which a certain experimentalist appears imperiously surveying his apparatus with one raised foot thrust firmly on top of a large dark rectangular box—said to be the coffin of Regge theory.) Of no avail; the physical world seemed to be in tune with Regge, and remained so for some time.

Then the reaction set in. The experimentalists, exploring all the different reactions they could think of, discovered behaviour that was sometimes not quite what was expected. The theorists, probing the intricacies of the argument, found all sorts of problems in going from the non-relativistic to the relativistic situation. Hundreds of papers were written, spawning a rich and exotic vocabulary. Trajectories could bear 'daughters'; trajectories might 'conspire' together; 'ghost-killing' mechanisms would have to operate at certain 'nonsense' points. And so on.

Many of these points have never been resolved fully, but what emerges as the main difficulty in Regge theory can be understood heuristically as follows.

Quantum field theory taught us, long ago, to regard scattering processes as 'mediated' by the exchange of 'virtual' particle, with some well-defined spin, a π -meson say, with zero spin,



and bequeathed to us the impossible task of summing up the effects of exchanging these virtual particles in all possible ways.



The expression given by Regge Theory, drawn symbolically as



miraculously represents, at high energies, the sum of a huge subset of these diagrams, and can be thought of as representing the exchange of a composite object a 'Reggeon' with variable spin the rôle of the spin being played by the ubiquitous $\alpha(t)$.

But though infinitely large, this subset does not, alas, exhaust all the diagrams, and we are forced to grapple with multi-Reggeon exchange diagrams such as



The theory becomes difficult and subtle—a new form of field theory—but at least one relieved of some of the worst idiosyncrasies of ordinary field theory. For example, in ordinary field theory it is impossible to allow the exchange of a particle whose spin is greater than one unit, because doing so triggers the appearance of unmanageable infinities in the calculations. But Reggeons with arbitrarily high spin can be exchanged with equanimity.

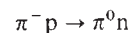
Reliable results for diagrams with multi-Reggeon exchange are hard to come by. It does seem, though, that adding in the results of multi-Reggeon exchange gives answers substantially different from the single Regge pole results. If so, what on earth does the good agreement between some experiments and single Regge pole exchange mean?

That is the present conundrum. Why do some experiments support Regge poles? Why do others disagree? And is the disagreement in a direction that could be explained by multi-Reggeon exchange?

In the past few years there have been two shifts of emphasis in the experimental work.

(1) Improved techniques of detection and the availability of more intense beams of elementary particles from the new accelerators have made it possible to study more refined aspects of collision processes. Rather than simply asking with what frequency particle C emerges at angle θ in the reaction $A + B \rightarrow C + D$, one can now ask also about its 'polarisation' (the direction of its spin), or even about the correlation between the direction of its spin and that of D .

Regge pole theory makes very precise predictions for these measurements—and they are generally wrong. A marvellous example is the reaction



in which the colliding positively charged proton and negatively charged π -meson effectively swap electric charges, emerging as an electrically neutral π -meson and neutron. It can be demonstrated quite rigorously that one, and only one, kind of Reggeon can be exchanged in this process. Thus the usual complication arising from the adding of the effects of several Reggeons is absent and the predictions of pure Regge pole theory are laid bare, open, one might have believed, to the ultimate judgement.

The measured angular distribution of the emerging particles and the manner in which this changes with energy provide a brilliant success for the theory. Even the shape of the angular distribution (see Fig. 1) with its changing sharp minimum is understood as a consequence of the structure of the theory. (The trajectory involved is that of the ρ -meson family and the point at which the minimum occurs corresponds to the value of t at which

$\alpha_p(t) = 0$). But the spin of the neutron, predicted quite firmly to be orientated randomly, with no specially preferred directions, has now been found to point predominantly in one particular direction (it is polarised) thus dealing a grievous blow to the theory.

Another example of great interest is the pair of reactions

$$\pi^+ p \rightarrow \pi^+ p$$

$$\pi^- p \rightarrow \pi^- p$$

involving the collision of π -mesons of positive or negative electric charge with protons. For reasonably small angles of scatter one expects little difference between the two reactions, and this is confirmed experimentally, but for large angles of scatter (close to backward scattering) Regge theory predicts significantly disparate behaviour. The reason is simple. In the reaction $\pi^- p \rightarrow \pi^- p$, at large angles of scatter, the only Regge pole of importance is that belonging to the family of nucleon resonances usually labelled Δ (the first member is the spin 3/2, isotopic-spin 3/2 resonance with mass 1,236 MeV), so the dependence upon energy in backward $\pi^- p \rightarrow \pi^- p$ is proportional to E raised to the power α_Δ . On the contrary, in the reaction $\pi^+ p \rightarrow \pi^+ p$ at backward angles, two Regge poles play a part, one belonging to the Δ family and the other to the family of the nucleon itself (labelled N), and their effects must be added. Are they equally important? The shape of the angular distribution in $\pi^+ p \rightarrow \pi^+ p$ (see Fig. 2), with its very sharp minimum close to point of completely backward scattering ($\theta = 180^\circ$), would emerge naturally from the theory if the N Regge pole dominated. (The dip would just correspond to the point at which $\alpha_N(t) = 0$). Our conclusion: at medium energies, where the processes have so far been measured, the N Regge pole dominates, and since it only

Fig. 1 The angular distribution in $\pi^- p \rightarrow \pi^0 n$ at several energies⁴, $\blacktriangledown$, CERN, 1968 5 GeV; Δ , CERN, 1965 10 GeV; $\blacksquare$, HEP, 1974 21 GeV; $\circ$, Fermilab, 1974 40.6 GeV; $\bullet$, Fermilab, 1974 101 GeV; $\square$, Fermilab, 1974 200 GeV.

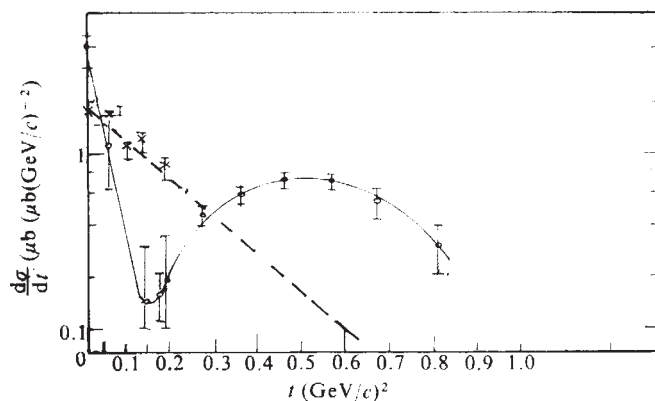
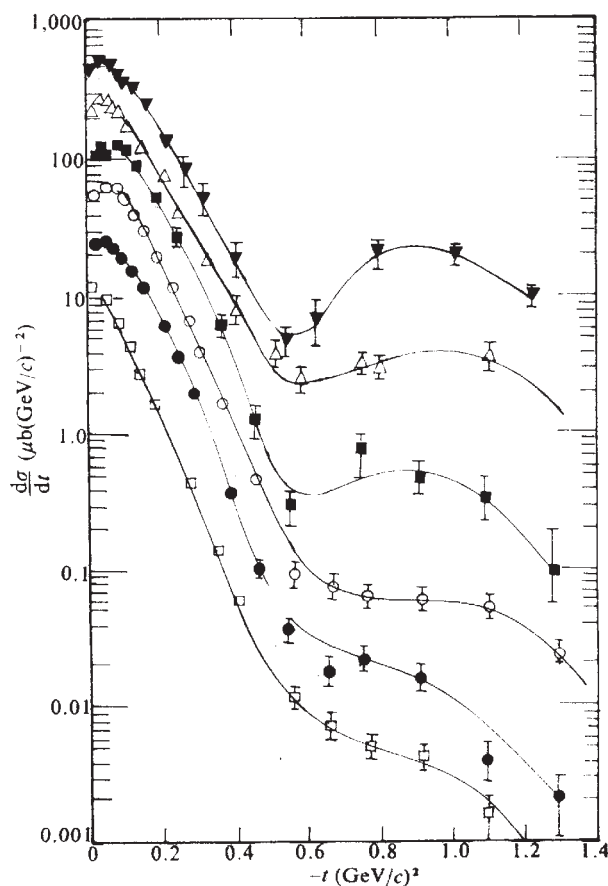


Fig. 2 The angular distributions for $\pi^- p \rightarrow \pi^- p$ (---) and $\pi^+ p \rightarrow \pi^+ p$ (—) near the backward direction (eye fits)⁵.

contributes to one of the reactions, that is, to $\pi^+ p \rightarrow \pi^+ p$, we have a natural explanation for the striking difference between the angular distributions of the two reactions (Fig. 2).

But now comes the rub. From the properties of the two families of particles, N and Δ , we know that α_Δ must be numerically greater than α_N . Thus although at present energies the N contribution dominates in $\pi^+ p \rightarrow \pi^+ p$ this cannot continue to be true at higher energies. For the Δ contribution is growing like E^{α_Δ} , compared with E^{α_N} for the N, and is bound to overhaul the N and eventually dominate at very high energies.

We thus have a dramatic prediction for very high energies. Both reactions $\pi^+ p \rightarrow \pi^+ p$ and $\pi^- p \rightarrow \pi^- p$ will be dominantly controlled by the same Regge pole Δ . Their angular distributions, so dissimilar at medium energies, will then look alike, and will take on the shape at present shown by $\pi^- p \rightarrow \pi^- p$.

A splendid and challenging prediction! But preliminary results at fairly high energies (about 70 GeV) show no change at all. The $\pi^+ p$ angular distribution is just as sharp and spiky as it ever was. Very careful measurements are planned at Fermilab at energies of 100–200 GeV. Their outcome is eagerly awaited.

As a last and most mysterious example consider neutron-proton collisions in which the neutron bounces back at very large angles. Although the experiments are difficult (because it is hard to know exactly how many neutrons are arriving per second in one's beam) it does seem to be established that the energy dependence in this reaction is most peculiar. It starts off at low and medium energies behaving as if it was controlled by the trajectory α_π belonging to the Regge family of the π -meson. As α_π is approximately equal to zero, E^{α_π} gives hardly any change at all as the energy increases. But suddenly, at energies in the region of 60 GeV, it begins to grow. Now some growth with energy is not at all surprising. In fact, it is predicted, because the trajectory α_ρ of the ρ -meson Regge family, should also play a part, and its numerical value is about $\frac{1}{2}$, yielding a contribution increasing like $E^{1/2}$. But the growth discovered experimentally seems to be far more rapid; some would even claim it proportional to E itself.

If this were to be substantiated it would really upset the apple-cart—for the number one, to which power it is claimed E is raised, is in Regge theory somewhat sacrosanct. It is the attribute only of that mysterious trajectory, the 'Pomeron', which so far as we know, is not connected with any family of particles or resonances, and which possesses the virgin properties of the vacuum itself—no electric charge, no baryon number, no isotopic spin, no strangeness.

But the Pomeron's role is limited to 'diffractive' processes: elastic reactions like $pp \rightarrow pp$ or $np \rightarrow np$ in which the projectile is scattered through a very tiny angle. In our example of large angle or backward scattering it is impossible to exchange a Pomeron. Whatever we exchange must be electrically charged and have a non-zero value of isotopic spin.

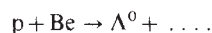
What should we conclude? Are the experiments wrong? Or if they are correct, are we discovering the existence of pretenders to the Pomeron throne? Is there perhaps a family of privileged Regge poles, with trajectories like the Pomeron's, but with different sets of charges, isotopic-spins and so on? (We have, in a moment of fantasy, already speculated upon the existence of such objects—and called them 'Odderons'⁶). Or, finally, is the unexpected energy dependence simply a signal of the failure of Regge pole theory?

(2) The existence of very high energy beams of particles has stimulated interest in reactions in which many particles are produced in the collision:



It is not generally possible to identify and analyse the properties of all the particles emerging, so, often, attention is focussed upon just one of them. (Measurements of this type are rather inappropriately referred to as 'inclusive' experiments.) A very clever argument of A. Mueller⁷ shows that many of the properties of inclusive experiments ought to be controlled by the same Regge poles that are active in the simpler reactions we were considering earlier. Many measurements, principally of the angle and momentum of one of the emerging particles in inclusive experiments, have agreed nicely with the predictions, and have yielded Regge trajectories in good agreement with those determined from simple scattering processes. But recently, a new phase in the experiments, in which the spin of the particle is also measured, has begun to provide some surprising results in contradiction with Regge pole theory.

The best example is probably one in which protons collide with a Beryllium target at 300 GeV to produce neutral lambda particles (Λ^0) plus much debris that is ignored:



Normally it is exceedingly difficult to determine the direction of a fast particle's spin, but it is a peculiar favour of Nature that when the Λ^0 , which is unstable, decays into a proton and a π^-

meson, one can determine the spin direction which the Λ^0 had from an elementary study of the angles of emergence of these decay products. (The Λ^0 is said to be 'self-analysing'.) According to Regge pole theory the Λ^0 should not be polarised. Experimentally, however, there is a definite favouring of one particular direction for the spin.

Where, finally, do we stand?

As regards Regge's own contribution, the introduction of the concept of complex angular momentum into quantum mechanics, that, as we have said, is inviolate. But the generalisations to the relativistic domain of elementary particle physics are fraught with difficulty and have a mixed record of success and failure. Many indeed are the predictions of angular distributions and their change with energy that have been confirmed experimentally, but many too are the cases of disagreement.

Given this state of affairs, is it conceivable that Regge pole theory is correct? In its simplest, naive form, with the exchange of single Reggeons, there is no hope. In its broader guise, with multi-Reggeon exchange, we simply do not know the answer. Sometimes it looks as if results are in the right direction but the calculations are too difficult, and any confrontation between experiment and these calculations could just as well reflect inaccuracy in our approximations as some basic fault of the theory.

Strangely though, despite these uncertainties, and even if the theory may be wrong, the language of the theory is likely to survive for a long time to come. For, stripped of all its details, Regge pole theory does focus upon a link between the behaviour of scattering and the quantum numbers of the 'object' that can be exchanged. And this, at a qualitative level, does seem to be a correct and useful concept. But the elucidation of the detailed dynamical properties of this 'object' will have to await a new Regge.

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articles

Reflection of X rays by neutron star surfaces

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A neutron-star surface should act as a good reflector for photons up to hard X-ray energies, due to the high density of the magnetic surface matter. Reflectivities are high, especially in spectral bands above the plasma and cyclotron frequencies depending on the photon polarisation. X-ray reflection may be important for the beaming of the binary neutron star Her X-1 and similar objects.

X RAYS are reflected under glancing angles by flat surfaces of condensed matter. This phenomenon is based on the fact that, for X-ray energies away from absorption edges the electrons can be considered as free particles such that the photons interact with them coherently. At frequencies ω larger than the plasma fre-

quency $\omega_p = (4\pi e^2 n_e / m_e)^{1/2}$, the refractive index $n = \{1 - \omega_p^2 / \omega^2\}^{1/2}$ is real and smaller than unity. Consequently, total external reflection occurs at glancing angles $\alpha < \alpha_c = \cos^{-1} n$. For terrestrial materials $n_e \sim 10^{24} \text{ cm}^{-3}$ and α_c is $\sim 2^\circ$ for $\sim 1 \text{ keV}$ photons.

According to current concepts (see, for example, ref. 1) a neutron star surface consists of a solid or liquid magnetic polymer. With respect to its reflective properties two radical changes occur in comparison with terrestrial matter. First, the surface electron density is $\propto B^{6/5}$ and will be of the order $\sim 10^{28} \text{ cm}^{-3}$ for $B \sim 10^{12} \text{ G}$ fields². This leads to a plasma frequency which is ~ 100 times larger than for normal matter. Consequently, glancing reflection should occur up to $\sim 100 \text{ keV}$ at 2° , or up to much larger angles at lower energies.

Second, the electron plasma is highly anisotropic since the

electron can move freely only along the magnetic field lines while their motion is quantised in the transverse direction. Consequently, the refractive and reflective properties of the surface will strongly depend on the magnetic field orientation and on the electron cyclotron frequency $\omega_B = eB/m_e c$.

Both effects depend crucially on the surface magnetic field strength, which is now known³ with some precision for the neutron star Her X-1 ($\sim 5 \times 10^{12}$ G). This fact and the possible effects of X-ray reflection on the beaming of this or other X-ray sources led us to investigate this problem quantitatively. Unfortunately, nothing is known about the macroscopic surface structure of neutron stars. But, it seems reasonable to assume that it is smooth enough to exhibit at least some mirror qualities.

We calculate here the X-ray reflectivity of a neutron star surface as function of photon energy, polarisation, and angle of incidence under idealised conditions. We also discuss possible deviations expected to occur in the case of real neutron star surfaces and summarise some expected observational effects which may have a role for systems like Her X-1.

X-ray reflection at a neutron star surface

We assume a plane surface which represents a sharp transition from vacuum to the high density surface matter. The magnetic field is orientated perpendicular to the surface. The latter will be a good approximation in the vicinity of the accretion column at a neutron-star magnetic pole. As already discussed, the X-ray optical properties of the surface will depend on the electron plasma frequency

$$\hbar\omega_p = (4\pi e^2 n_e / m_e)^{1/2} \hbar \quad (1)$$

and on the electron cyclotron frequency

$$\hbar\omega_B = eB/m_e c \hbar = 11.6 \times B_{12} \text{ keV} \quad (2)$$

The total number density of electrons can be calculated from the nuclei density of the condensed magnetic matter², which results in

$$n_e = 1.24 \times 10^{27} Z_{26}^{2/5} B_{12}^{6/5} \text{ cm}^{-3} \quad (3)$$

Assuming that the surface consists of iron we get for the plasma frequency

$$\hbar\omega_p = 1.45 \times B_{12}^{3/5} \text{ (keV)} \quad (4)$$

We neglect here the atomic binding energies of the electrons which is justified by the fact that for $B \sim 5 \times 10^{12}$ G and iron nuclei most electrons have binding energies smaller ~ 2 keV (W. Hillebrandt, personal communication).

In order to calculate reflectivities we follow the classical approach and use the boundary conditions which must be satisfied on the surface by the electromagnetic fields (see ref. 4). Besides the incident and reflected waves we have to consider two

refracted waves as two modes of propagation, the ordinary and the extraordinary one, exist in the magnetised plasma.

Figure 1 depicts the coordinate system and the notation used. The magnetic field is orientated perpendicular to the surface which is given by the plane $z = 0$, $y = 0$ is the plane of incidence. We neglect the effects of vacuum birefringence in a strong magnetic field^{5,6} and assume an index of refraction $n_0 = 1$ above the surface. This is justified because for a 10^{12} G field the deviations from unity are of the order 10^{-5} . Below the surface the dielectric properties are described by a tensor ϵ . The different waves are characterised by their electric vectors $\mathbf{E}$ and wave vector $\mathbf{k}$.

We first consider the properties of the two refracted waves. The refraction indices for the ordinary and extraordinary mode of propagation are given by the dispersion relation (refs 7 and 8):

$$\begin{bmatrix} S - n_m^2 \cos^2 r_m & -iD & -n_m^2 \sin r_m \cos r_m \\ iD & S - n_m^2 & 0 \\ -n_m^2 \sin r_m \cos r_m & 0 & P - n_m^2 \sin^2 r_m \end{bmatrix} \begin{bmatrix} E'_{xm} \\ E'_{ym} \\ E'_{zm} \end{bmatrix} = 0 \quad (5)$$

$$\text{with } R = 1 - \frac{\omega_p^2}{\omega^2} \frac{\omega}{\omega - \omega_B + i\omega_D}$$

$$L = 1 - \frac{\omega_p^2}{\omega^2} \frac{\omega}{\omega + \omega_B + i\omega_D}$$

$$P = 1 - \frac{\omega_p^2}{\omega^2} \frac{\omega}{\omega + i\omega_D}$$

$$S = \frac{R+L}{2}$$

$$D = \frac{R-L}{2}$$

where ω_p , ω_B , and ω_D are the plasma frequency, the cyclotron frequency and the damping frequency, respectively, and the index $m = 1, 2$ describes the ordinary and extraordinary mode. From this dispersion relation we derive the following amplitude ratios:

$$\frac{E'_{mx}}{E'_{mz}} = \frac{P - n_m^2 \sin^2 r_m}{n_m^2 \sin r_m \cos r_m} = \alpha_m \quad (6)$$

$$\frac{E'_{my}}{E'_{mz}} = \frac{-iD \alpha_m}{S - n_m^2} = \beta_m \quad (7)$$

and, as the determinant must vanish, the quartic equation for the two refractive indices:

$$An^4 - Bn^2 + C = 0 \quad (8)$$

with $A = S \sin^2 r_m + P \cos^2 r_m$

$$B = RL \sin^2 r_m + PS \cdot (1 + \cos^2 r_m)$$

$$C = PRL$$

For propagation modes, both the ordinary and the extraordinary ones, refraction is described by Snell's law

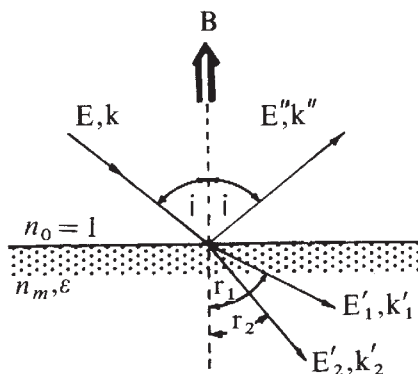
$$n_m = \sin i / \sin r_m \quad (9)$$

in which n_m implicitly depends on r_m .

The angle of refraction r_m can be calculated if we replace n_m in (8) by (9) and solve for $\sin r_m$. Inserting these values into (6) and (7) we find the amplitude ratios α_m and β_m of the electric field components below the surface.

In order to determine the electric field components of the reflected wave we use the boundary conditions at the surface which read in the present case:

Fig. 1 The propagation vectors for refraction and reflection at the plane surface.



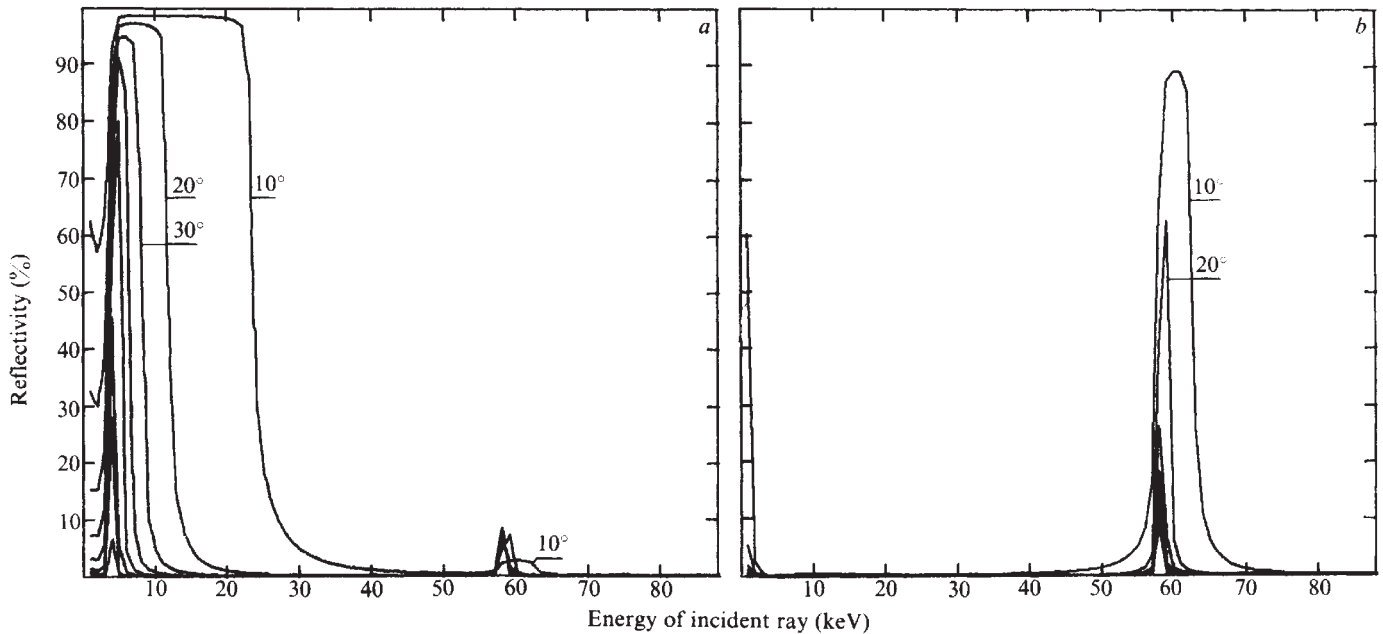


Fig. 2 X-ray reflectivities as function of photon energy for different polarisations of the incoming photon and grazing angles between 10° and 80° . Plasma frequency, 4 keV; cyclotron frequency, 58 keV; damping frequency, 0.1 keV. *a*, Polarisation parallel to plane of incidence; *b*, polarisation perpendicular to plane of incidence.

$$\left[\mathbf{E} + \mathbf{E}'' - \sum_{m=1}^2 \mathbf{E}'_m \right] \cdot \mathbf{Z}_0 = 0 \quad (10)$$

$$\left[\mathbf{k} \times \mathbf{E} + \mathbf{k}'' \times \mathbf{E}'' - \sum_{m=1}^2 \mathbf{k}_m' \times \mathbf{E}_m' \right] \cdot \mathbf{Z}_0 = 0 \quad (11)$$

$$\left[\mathbf{E} + \mathbf{E}'' - \sum_{m=1}^2 \mathbf{E}'_m \right] \times \mathbf{Z}_0 = 0 \quad (12)$$

$$\left[\mathbf{k} \times \mathbf{E} + \mathbf{k}'' \times \mathbf{E}'' - \sum_{m=1}^2 \mathbf{k}_m' \times \mathbf{E}_m' \right] \times \mathbf{Z}_0 = 0 \quad (13)$$

where $\mathbf{Z}_0$ is the unit vector in z -direction.

Representing $\mathbf{E}$ and $\mathbf{E}''$ by their components perpendicular and parallel to the plane of incidence, one gets the linear equations:

$$\begin{bmatrix} E_\perp \\ E_\parallel \\ E_\perp \\ E_\parallel \end{bmatrix} = \begin{bmatrix} \beta_1 & \beta_2 & -1 & 0 \\ \frac{n_1 \cos r_1 \beta_1}{\cos i} & \frac{n_2 \cos r_2 \beta_2}{\cos i} & 1 & 0 \\ \frac{\alpha_1}{\cos i} & \frac{\alpha_2}{\cos i} & 0 & 1 \\ \frac{P}{\sin i} & \frac{P}{\sin i} & 0 & -1 \end{bmatrix} \cdot \begin{bmatrix} E'_{1z} \\ E'_{2z} \\ E'_\perp \\ E'_\parallel \end{bmatrix} \quad (14)$$

Inverting this system of linear equations gives the dependence of $E_\perp$ on E . The corresponding reflectivities $|E''|^2/|E|^2$ and $|E''|^2/|E_\parallel|^2$ as a function of photon energy are shown in Figs 2 and 3, where we have replaced the angle of incidence i by the grazing angle $\pi/2 - i$.

For the cyclotron frequency the value measured for Her X-1 (58 keV, ref. 3) is used. The plasma frequency was calculated for

the corresponding magnetic field strength (5.3×10^{12} G) using equation (4), which leads to $\hbar\omega_p \sim 4$ keV. The damping frequency in intense magnetic fields has been treated in ref. 9. It turns out that in the present case $\hbar\omega_D$ is of the order 10 to 100 eV, that is small compared to X-ray photon energies. To improve the legibility of the figures in the vicinity of the resonances we arbitrarily used for our calculations a value $\hbar\omega_D = 100$ eV.

Figures 2 and 3 show that for photons polarised in the plane of incidence the reflectivity is near unity in a spectral band between $\hbar\omega_p$ and a cut-off energy depending on the grazing angle. Total external reflection ($n < 1$) takes place in this region. At the cyclotron frequency a small peak occurs which corresponds to the excitation of the extraordinary mode in the surface.

For photons polarised perpendicular to the plane of incidence one finds substantial reflectivity below the plasma frequency and in a spectral band between $\hbar\omega_B$ and a cut-off energy depending again on the grazing angle. Also in this upper energy band the high reflectivity is due to total external reflection ($n < 1$). We note that both spectral bands in which total external reflection occurs are limited by very steep slopes on both sides. If one plots the reflectivity as function of the grazing angle, as shown in Fig. 4, one finds a steep cut-off behaviour here as well.

Application to Her X-1

According to the generally accepted model for Her X-1, the pulsed X rays originate from hot spots at the magnetic poles on the stellar surface which are heated by the accreted material falling down along the polar field lines. It is clear that a beaming mechanism is required in order to account for the sharpness of the observed 1.24-s X-ray pulses, however, there is no agreement regarding the beaming geometry. The basic possibilities discussed are pencil beams (along the polar field lines) or fan beams (perpendicular to the field lines). We regard the fan beam geometry as the more likely possibility since the radiation most easily escapes sideways from the base of the accretion funnel. In this case part of the radiation will hit the stellar surface where it undergoes reflection, as discussed above. The reflected component should lead to sharp structures in the spectral time variability of the source due to the sharp on-sets and cut-offs in reflectivity as functions of grazing angle and photon energy.

Observations show that the 1.24-s pulsations of Her X-1 are characterised by: a double peak structure at low energies (~ 2 to ~ 20 keV, refs 10, 11); single peaks between ~ 20 keV and

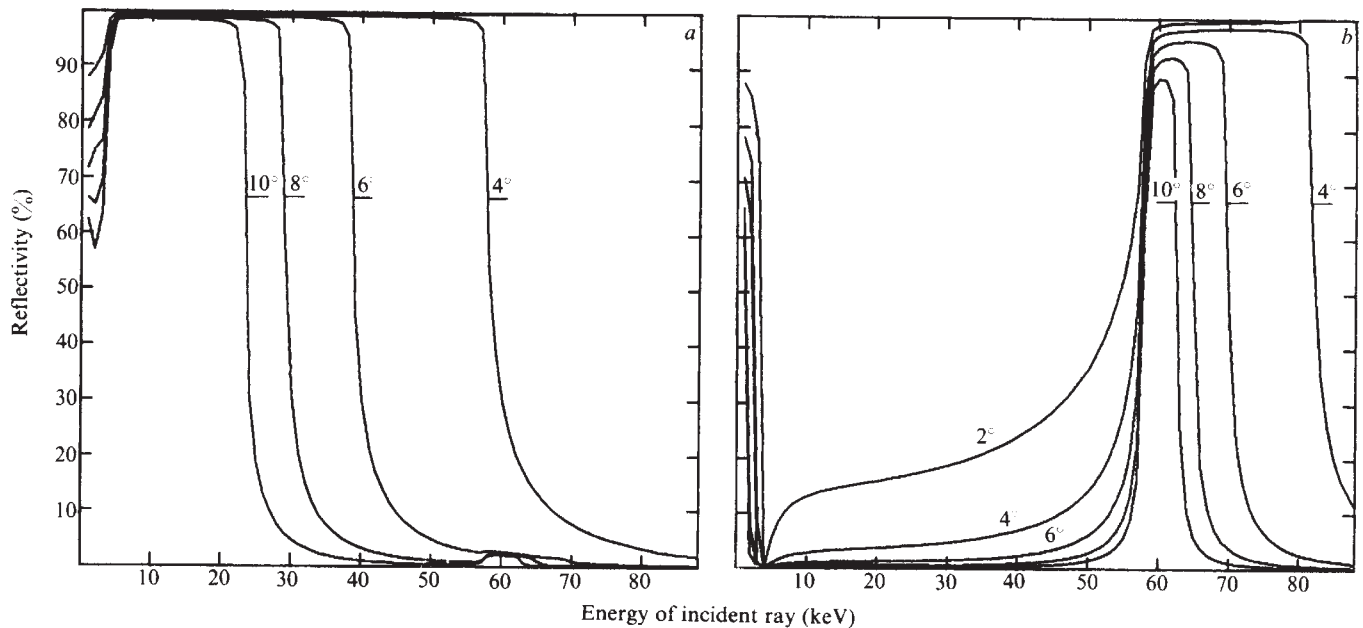


Fig. 3 Same as Fig. 2; grazing angles 4° to 10° .

~ 60 keV, including the cyclotron resonance at ~ 58 keV (ref. 12); and a double peak structure between ~ 60 keV and 90 keV, that is above the cyclotron resonance (ref. 12).

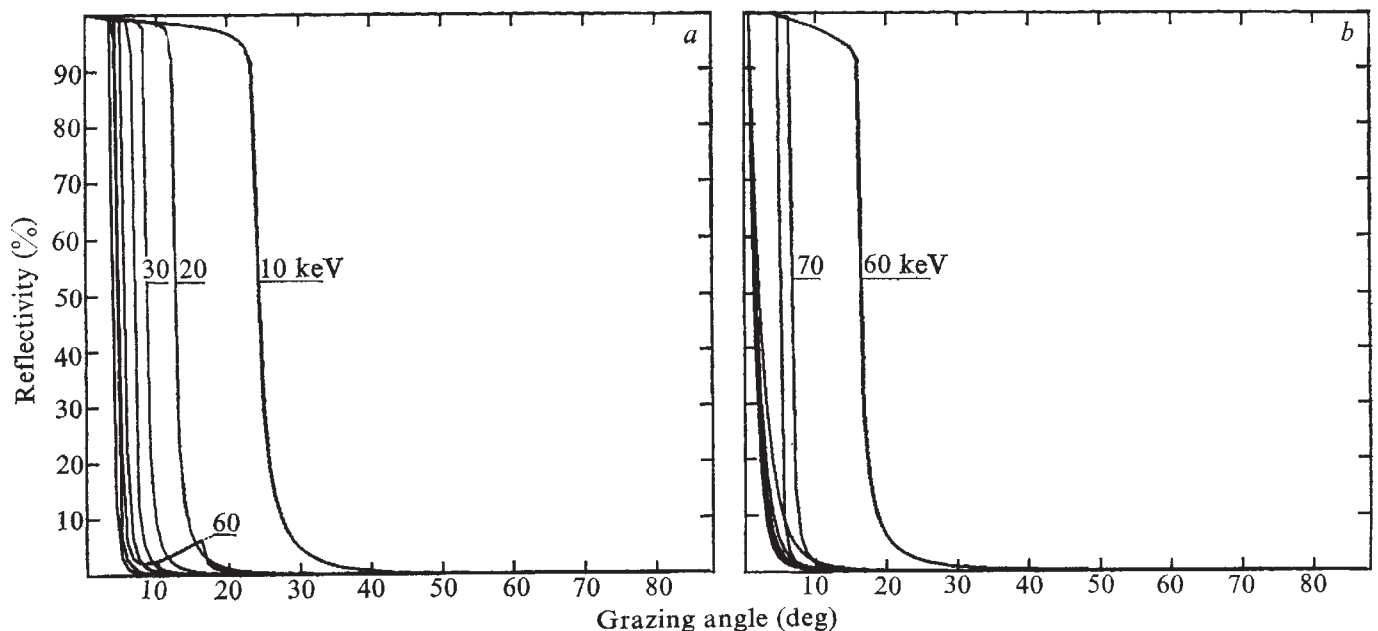
This complex behaviour can be explained by the following simple model. Between 20 and 60 keV one sees the direct, unmodified fan beam which is emitted from the base of the accretion column under a polar angle $\sim 1/2$. This requires that near pulse maximum the line of sight is approximately at right angles to the polar field lines. In the 2–20 keV range and above the cyclotron resonance at 58 keV, an additional reflected beam occurs which has its intensity maximum at $\theta_m < \pi/2$. As the line of sight crosses the reflected beam twice in one rotational period, namely just before and after reaching the fan beam maximum, double pulses are produced which are superimposed on single pulses.

According to Figs 2 and 3 the spectrum of the reflected beam will strongly depend on the polarisation of the incident beam. This polarisation, however, is expected to be zero, as any

polarisation of the X-ray beam emerging from the accretion column will be completely destroyed as the beam propagates through the strong magnetic field near the neutron star due to vacuum birefringence⁶. Therefore, the shape and spectrum of the reflected beam will depend only on the shape and spectrum of the fan beam, and not on its polarisation properties at emission which may be very complex.

Our considerations will not be altered substantially if we drop the assumption of a plane mirror with perpendicular magnetic field made above and consider the spherical polar cap of a neutron star with a dipolar magnetic field. A considerable deterioration of specular reflection, however, will be caused if an atmosphere exists which is dense enough to scatter the photons incoherently before they reach the high density surface. The answer to the question of whether such an atmosphere exists will depend on the cohesive energy of surface matter and on the surface temperatures. At 5×10^{12} G the cohesive energy of the linear chains which form the condensed matter is ~ 8 keV (ref. 2). In the immediate

Fig. 4 X-ray reflectivities as function of grazing angle for different energies and polarisations of the incoming photons. *a*, Polarisation parallel to plane of incidence; *b*, polarisation perpendicular to plane of incidence.



surroundings of the radiating accretion column the surface temperature may be above this value due to heating by the refracted photons and Thomson scattering recoils. Also heat conduction in the stellar material may play a role in this context. One, therefore, expects that up to a certain radial distance from the accretion column the neutron star surface is covered by a dense atmosphere and represents a 'tarnished mirror', reflecting quasi-isotropically by Thomson scattering. Specular reflection will occur in a ring zone between this region and the neutron star horizon as seen from the radiation source.

Finally, the Thomson scattered component may account for the off-pulse emission of Her X-1 which has a spectrum similar to that of the pulsed component. Another source of the diffuse off-pulse component may be the scattering by surface micro-roughness or Bragg reflection by the one-dimensional lattice of the magnetic polymer constituting the surface matter. A more detailed model for the complex spectral variability of Her X-1 will be discussed in a separate paper.

After writing this paper we became aware of ref. 13, which contains an alternative and more general treatment of the electrodynamic problem of 'reflection and refraction of electromagnetic waves on plasma in homogeneous magnetic fields'. This problem has also been discussed in ref. 14 in the context of emissivity of magnetic neutron star surfaces.

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Rb-Sr mantle isochrons and variations in the chemistry of Gondwanaland's lithosphere

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Isotopic data for the Mesozoic tholeiites of Gondwanaland are used to evaluate theories explaining the chemistry of mantle-derived rocks; a crustal contamination hypothesis is discarded. These findings may contribute towards an understanding of crust-mantle evolution.

BECAUSE of the potential importance of Rb-Sr mantle isochrons^{1,2} in models of igneous petrogenesis, we have examined the published isotopic data for the Mesozoic tholeiites of Gondwanaland. These tholeiites were chosen because they are the product of roughly synchronous magmatism distributed over a vast surface area and they share a similar mode of occurrence and major element chemistry. Thus they can be used to appraise the question of primary versus contaminated chemistry in mantle-derived rocks and also the possibility that plateau-type tholeiites contain information on the local and regional heterogeneities in Earth's mantle.

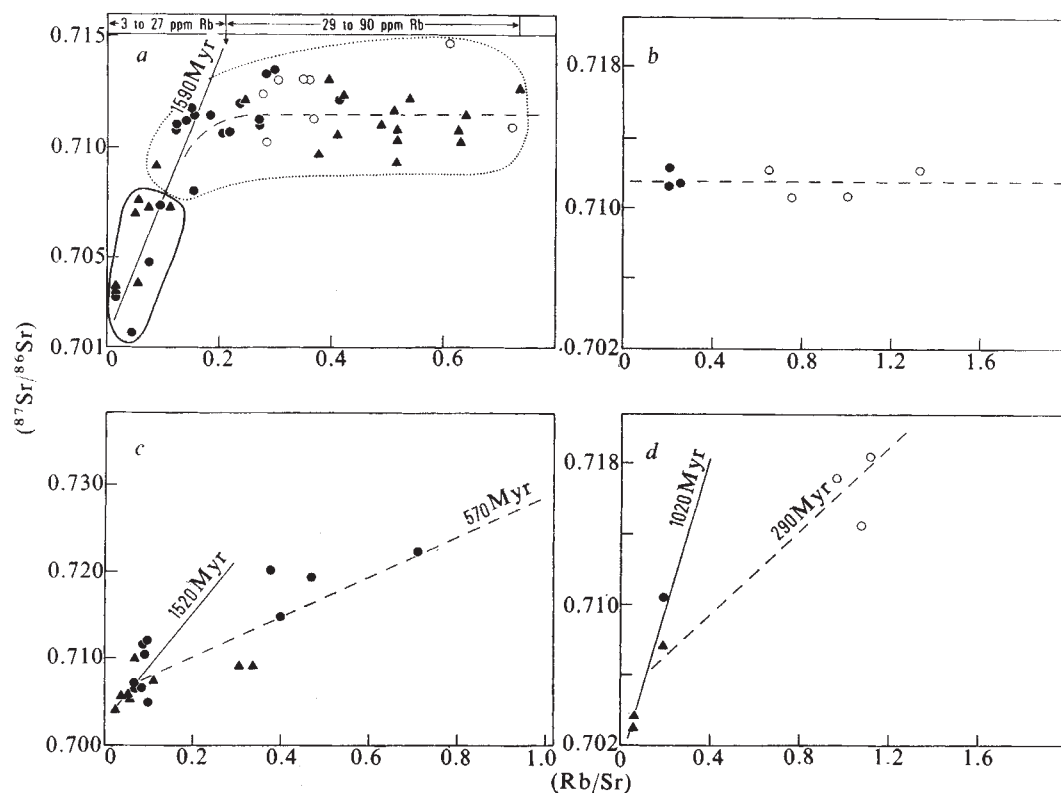
Mantle isochron properties

All available Rb/Sr and Sr-isotopic data for these tholeiites have been retrieved from the literature, grouped according to geographic distribution both within and between the individual post-Gondwanaland continental fragments, and examined for Rb-Sr mantle isochron properties. We have corrected the retrieved ⁸⁷Sr/⁸⁶Sr ratios for their post-crystallisation component by applying a blanket 160 Myr age correction. The Rb/Sr of the samples is such that real variation in this correction age has little influence on the calculated initial ⁸⁷Sr/⁸⁶Sr values. The resulting initial ⁸⁷Sr/⁸⁶Sr versus Rb/Sr data for each association are presented in Fig. 1. Each plot includes the more felsic members of each association (for example, the pegmatites of the Ferrar Group; the Rio Grande do Sol felsics of the Serra Geral Formation; the Red Hill granophyres of Tasmania). Inspection of the diagrams reveals that good correlations occur for three of the five associations (Karoo, Serra Geral

and Queen Maud Land dolerites) while the remaining two associations (Ferrar Group and Tasmanian dolerites) define horizontal lines corresponding to a small range in initial ⁸⁷Sr/⁸⁶Sr for a wide range in Rb/Sr. Furthermore, for two of those associations showing correlations, rejection of the felsic members with high Rb/Sr leads to a correlation which, if interpreted as an isochron, corresponds to a much older age (Fig. 1c, d). The relevant statistical data for these correlations together with literature sources and number of samples are given in Table 1; the resulting best-fit lines are significantly different from zero at levels of confidence usually greater than 95%.

There are a variety of mechanisms which could produce correlations of the type observed in Fig. 1 and no obvious criteria to permit distinction between a crustal and mantle-related process. There is, however, considerable evidence against crustal contamination as the cause of these correlations. For the Mesozoic tholeiites such evidence would include lack of 'mixing line' relationships between all of the elements analysed, despite the thesis that the variability in isotopic composition is due to variable assimilation of that contaminant. This is well illustrated for the Kirkpatrick Basalts of Antarctica³, the proposed effusive phase of the Ferrar dolerites⁴. These basalts show initial Sr versus major element correlations for SiO₂ and total Fe, and a single contaminant is advanced to explain this correlation. However, the Sr-isotopic composition does not correlate with many of the other major elements (including Na and K) nor with Rb. The latter is higher in concentration than levels normally observed in basaltic magmas and any mixing-contamination explanations must satisfy the Rb observations. Other evidence would be an unlikely composition of supposed contaminant; any reasonable contamination mechanism will probably involve more than one rock composition; however, the average contaminant must correspond to some reasonable rock not too different in composition from that of the average crust. For the Kirkpatrick basalts, the proposed contaminant had a CIPW norm composition of:

Fig. 1 Rb/Sr versus initial $^{87}\text{Sr}/^{86}\text{Sr}$ for Mesozoic tholeiites. ●, Dolerites; ▲, basalts; ○, felsic members (such as dacites, granophyres); solid line mantle isochrons are for the less differentiated samples ($\text{Rb}/\text{Sr} < 0.2$, that is, $\text{SiO}_2 < 53\%$) and the dashed-line mantle isochrons are for all the data from each association (including felsic members if available). For literature sources see Table 1. *a*, Antarctica: Ferrar Group (including Kirkpatrick basalts) enclosed in dotted-line field; Queen Maud Land dolerites enclosed in solid-line field. The range of Rb values (p.p.m.) is included for reference. The solid-line mantle isochron is for the Queen Maud Land samples only. *b*, Australia: Tasmanian dolerites and granophyres. *c*, South Africa: Karroo dolerites and basalts (including samples from the Lebombo Nuanetsi Igneous Province). *d*, South America: Serra Geral Formation.



ferrosilite, 28%; quartz, 25%; albite, 19%; orthoclase, 18%; Na-metasilicate, 4%; diopside, 3%; ilmenite, 1%; apatite, 1%. While it is possible that mixtures of some crustal assemblages could produce a contaminant of this composition, it is highly unlikely that such a contaminant could exist in sufficient quantities to account for the wholesale contamination required by the model. Similar conclusions were reached by Weigand and Ragland⁵ in discussing the origin of the Mesozoic tholeiites of eastern North America.

The many other similar arguments would not strengthen our case against crustal contamination, but rather dilute it in prolixity. We thus use the isotopic data for the Mesozoic tholeiites to render the crustal contamination hypothesis untenable.

Isochrons for oceanic tholeiites

In oceanic regions, remote from contamination by continental crust, correlations of the type shown in Fig. 1 often occur².

Table 1 Rb-Sr mantle isochron data for Mesozoic tholeiite associations of Gondwanaland

Location	Association	MIRA	MIIR	<i>r</i>	<i>N</i>	Reference
ZONE A						
South America	Serra Geral Formation: all data (<i>b,d,d</i> ¹)	290 ± 50	0.7047 ± 14	0.93(3)	7	9
	data with Rb/Sr < 0.2 (<i>b,d</i>)	1,020 ± 280	0.7011 ± 16	0.93(4)	4	9
South Africa	Karoo System: all data (<i>b,d</i>)	570 ± 90	0.7059 ± 10	0.56(2)	19(2)	25, 27, 28
	data with Rb/Sr < 0.2 (<i>b,d</i>)	1,520 ± 640	0.7031 ± 20	0.84(4)	13	25, 27, 28
Antarctica	Queen Maud Land: all data (<i>b,d</i>)	1,010 ± 420	0.7031 ± 11	0.63(1)	11(1)	4
ZONE B						
Antarctica	Ferrar Group: all data (<i>b,d,g,p</i>)	n.s.	n.s.	n.s.	40	25, 4, 3
	data with Rb/Sr < 0.2 (<i>b,d</i>)	n.s.	n.s.	n.s.	8	25, 4, 3
Australia	Tasmanian dolerites: all data (<i>d,g</i>)	n.s.	n.s.	n.s.	7	26
THOLEIITES						
	Gondwanaland average data plus oceanic tholeiites, excluding Serra Geral value	1,620 ± 55	0.7023 ± 1	0.98(4)	14	2, and above list

All ± values given at 1σ. Details of mantle isochron regression procedures given in ref. 1. MIRA, mantle isochron regression age (Myr); MIIR, mantle isochron initial ratio; *r*, Pearson correlation coefficient (the numbers in parenthesis are increments of the standard deviation and indicate the level of confidence at which the slope of the fitted regression line differs from zero); *N*, samples included in the regression (the numbers in parenthesis are the numbers of available samples excluded); *b*, basalt; *d*, dolerite; *d*¹, dacite or rhyodacite; *g*, granophyre; *p*, pegmatoid; n.s., not significant. All averaged data from Table 2.

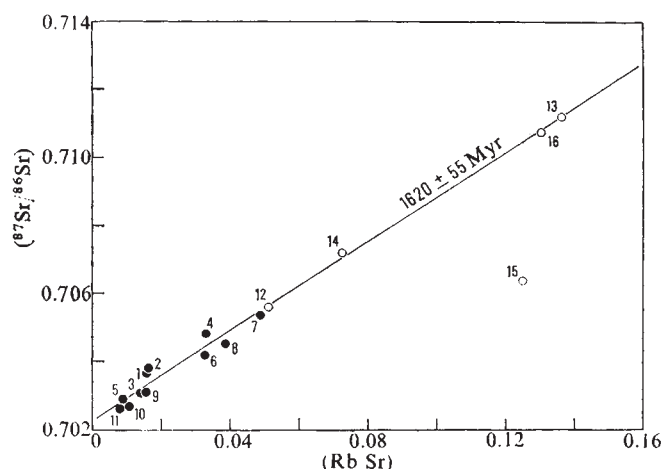


Fig. 2 Rb-Sr mantle isochron (solid line) for oceanic and Mesozoic tholeiites. Each point represents an average of available data on individual samples from either a given island, island group (●) or continental association (○). Numbers refer to the data index in Table 2.

For average island tholeiites these correlations correspond to an age of $1,600 \pm 200$ Myr, which is remarkably concordant with the Pb-Pb isochron ages of oceanic basalts ($1,800 \pm 100$ Myr). Because of this concordance between radiometric systems in which the systematics differ markedly during petrogenesis (especially during variable degrees of partial melting and differentiation), we argued that the Rb/Sr data defined a mantle isochron dating a real event, possibly the separation of an isolated asthenosphere (and mesosphere) within a previously more uniform and convecting mantle. In arriving at the 1,600 Myr Rb-Sr mantle isochron for oceanic tholeiites, we averaged the data from each island or island group, excluding any differentiated samples which could have influenced the systematics. The same treatment has been applied to the Mesozoic tholeiites, where differentiation has played a significant part in their magmatic histories as revealed by major element compositions (for example, refs 6, 7). Because only a few isotopic analyses have accompanying major and trace element data we could not independently identify the more primitive samples. Examination of available major element, trace element and petrographic data, however, indicate that the Rb/Sr ratio is extremely sensitive to bulk chemistry and can be used in place of a major or trace element index of differentiation. It was found that a Rb/Sr ratio of about 0.2 corresponds to an SiO_2 level of approximately 53%, and hence samples with an Rb/Sr ratio of 0.2 or less have chemical compositions more like 'normal' basaltic tholeiite. It is recognised that samples with Rb/Sr less than this value could also be somewhat differentiated and we emphasise that the Rb/Sr cut-off of 0.2 is used merely to distinguish between the more and less differentiated tholeiites. The effects of this differentiation on the mantle isochron systematics is well illustrated by the Karroo and Serra Geral tholeiites. Rejection of samples with Rb/Sr > 0.2 leads to significantly older mantle isochrons in the range of 1,000 to 1,500 Myr (Fig. 1 and Table 1). Clearly, differentiation rotates the mantle isochrons to younger ages, thereby masking source-region age information.

The calculated average values for initial $^{87}\text{Sr}/^{86}\text{Sr}$ and Rb/Sr of the least differentiated samples from each association are listed with the modern oceanic tholeiite data in Table 2 and plotted in Fig. 2. The Mesozoic data plot along the extension of the oceanic tholeiite mantle isochron with the exception of the Serra Geral Formation, where weathering may have rendered the data suspect. Published data for the Tasmanian samples all have Rb/Sr > 0.2, and so were excluded from this plot, but unpublished data with Rb/Sr < 0.2 have been included in Fig. 2 (these data will be the subject of a separate paper). When treated together, the tholeiite data define a 1,620 Myr mantle isochron (Table 1) with a 1σ error

Table 2 Average Rb/Sr and initial $^{87}\text{Sr}/^{86}\text{Sr}$ for tholeiites from oceanic islands[†] ocean floors[†] and the Gondwanaland Mesozoic tholeiite associations (this paper)

Location	Rb/Sr	$^{87}\text{Sr}/^{86}\text{Sr}^*$	N [†]	Graph no. [‡]
Oceanic island tholeiites				
Bouvet	0.016	0.70369	4	1
Hawaiian Islands	0.017	0.7038	30	2
Iceland	0.014	0.70307	13	3
Kerguelen	0.033	0.7048	5	4
Kolbeinsey	0.009	0.70290	1	5
Reunion	0.033	0.7042	8	6
Samoa	0.049	0.7053	5	7
St Pauls	0.039	0.7045	7	8
Ocean floor tholeiites				
Indian	0.015	0.70314	8	9
Pacific	0.011	0.70265	15	10
Atlantic	0.008	0.70264	13	11
Gondwanaland tholeiites				
Queen Maud Land	0.051	0.7056	11(1)	12
Ferrar Group	0.136	0.7112	7(1)	13
Karoo System	0.072	0.7072	13	14
Serra Geral Fm.	0.125	0.7063	4	15
Tasmania	0.130	0.7108	4	16

*Relative to *E* and *A* standard value of 0.70800.

†N = number of samples averaged, value in parenthesis is number of available samples excluded from the average.

‡Reference number for Fig. 2.

Average data for least differentiated tholeiites (Rb/Sr < 0.2).

(± 55 Myr) comparable to that of geochronological-type isochrons used for standard stratigraphic purposes. This alignment of oceanic island and Mesozoic tholeiite data along a common isochron would seem to require an exceptional coincidence if crustal contamination is the reason for the high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the Mesozoic tholeiites. We consider this to be significant evidence against crustal contamination as a cause for the elevated and variable Sr isotope ratios in these tholeiites. It is also contradicts previous arguments for the bulk assimilation of crustal material in their petrogenesis, and encourages the interpretation of the isotope compositions of these rocks in terms of primary mantle properties.

Gondwanaland regional variations

When the five associations studied in this paper are plotted on a reconstructed Gondwanaland⁸, they define two geographic

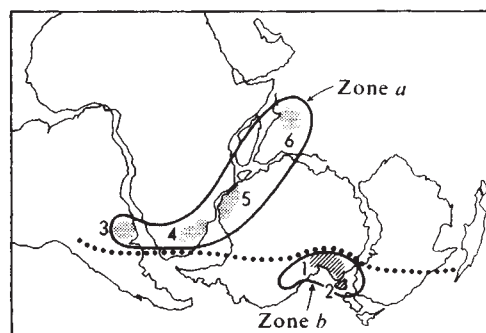


Fig. 3 Location of Mesozoic tholeiite associations plotted on Smith and Hallam's⁸ reconstruction of Gondwanaland. The continental interior margin of Du Toit's²⁴ Samfrau geosyncline is shown (heavily dotted line). Zone *a* includes Mesozoic tholeiites that have traversed stable cratonic interiors of the continents while Zone *b* includes those tholeiites that have traversed cratonic-margin regions. 1, Ferrar Group including the Kirkpatrick basalts; 2, Tasmanian dolerites of south-east Australia; 3, Serra Geral Formation, South America; 4, Karroo dolerites including the Lebombo-Naunetsi Igneous Province, South Africa; 5, Queen Maud Land dolerites of Antarctica; 6, Deccan Traps, India.

provinces (labelled zones *a* and *b*, Fig 3). These two provinces differ in several ways. Zone *a* tholeiites have traversed ancient basement rocks (2,000 Myr or older; refs. 9–13) constituting the cratons of each post-Gondwanaland fragment, while zone *b* tholeiites have been emplaced in a cratonic margin region dominated by late Precambrian and Palaeozoic orogenesis (basement ages in the range 400 to 1,000 Myr; refs. 14–17). Zone *a* has 'normal' surface heat flux values typical of cratonic regions while zone *b* is characterised by heat flow values about twice those of zone *a*^{18, 19}. Zone *a* tholeiites show mantle isochrons, zone *b* tholeiites do not. Zone *a* tholeiites show lower average ⁸⁷Sr/⁸⁶Sr ratios (0.7064) than zone *b* tholeiites (0.7112).

Considering these differences, we suggest that the lithosphere under zone *b* is significantly more enriched in radioactive heat producing elements than that underlying zone *a*. A relative enrichment in zone *b* lithosphere of K, Rb and U would lead to higher surface heat flow values and higher ⁸⁷Sr/⁸⁶Sr ratios. The lack of mantle isochrons in zone *b* tholeiites may reflect a relatively homogeneous or well-mixed lithosphere in this region. This suggests that zone *b* lithosphere may be composed dominantly of 'enriched' mesosphere-derived material relative to more depleted asthenosphere components. Also, high initial ⁸⁷Sr/⁸⁶Sr ratios (0.71 or more) are pervasive in many mafic igneous rocks that have been emplaced in the Australian sector of the Samfrau Geosyncline^{17, 20} (L. Black, personal communication, C. B., unpublished data) indicating a consistent regional anomaly in the source region corresponding to zone *b*. In Fig. 3, we have extended zone *a* to India because the Mesozoic tholeiites of that sub-continent (Deccan Traps) occur within a craton with basement of at least 2,500 Myr²¹. It could be predicted that, when data become available, the Deccan Traps will also show Rb–Sr mantle isochrons, although whether the accompanying range in initial ⁸⁷Sr/⁸⁶Sr resembles that of the Karroo and Serra Geral tholeiites or that of the Queen Maud Land dolerites awaits investigation. Based on our analysis of the Mesozoic tholeiites of Gondwanaland, a case can be made for the existence of gross heterogeneities in the underlying lithosphere manifest on all scales from local to continental, and for the development of those heterogeneities during an event perhaps associated with the separation of asthenosphere from mesosphere 1,600 to 1,700 Myr ago. Furthermore, we find that the cratonic margin part of Gond-

wanaland's lithosphere is probably much more enriched on average than the regional sub-cratonic lithosphere. It is not accidental that this enriched lithosphere correlates with areas of high heat flow, numerous anatectic granites^{22, 23}, high incidence of economic mineralisation of diverse types, and anomalous Sr-isotope composition in many mafic igneous rocks of Precambrian to Palaeozoic age. Indeed one may speculate that the reactivation of that sector of Gondwanaland during the late Precambrian and Palaeozoic was due primarily to the enriched nature of the underlying lithosphere. Clearly we have left many questions unresolved and there are many new ones provoked, but we are confident that investigations orientated to searching for and understanding Rb–Sr mantle isochrons will have a profound effect on our understanding of crust–mantle evolution.

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Structure of vancomycin and its complex with acetyl-D-alanyl-D-alanine

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Vancomycin, a broad-spectrum antibiotic, inhibits the growth of cell walls by complex formation with peptides terminating in D-alanyl-D-alanine. The structure of vancomycin was determined by X-ray analysis of the degradation product CDP-I. A model of the complex is proposed based on this study and spectroscopic data.

THE antibiotic, vancomycin, was first isolated in 1956 from cultures of *Streptomyces orientalis*¹. Although much studied, its full chemical structure could not be established. It is a broad-spectrum antibiotic but because of adverse side effects, its use is restricted to the treatment of staphylococcal infections (for example, septicaemia, pneumonia and wound infections) when

other antibiotics are ineffective. There are no naturally occurring strains of bacteria known to be resistant to vancomycin, nor do bacteria readily develop resistance to it. Clinical studies of vancomycin have been reviewed by Lightbown².

Vancomycin is known to act by inhibiting the biosynthesis of the cell-wall mucopeptide, leading to a cessation of growth and the eventual destruction of the cell by lysis. Perkins has shown^{3–5} that it forms complexes with the nucleotide-N-acetyl muramyl pentapeptide precursor, the reaction being specific for peptides with D-alanyl-D-alanine at the carboxyl terminus. Such complex formation either with the lipid intermediate or with uncross-linked peptidoglycan would inhibit mucopeptide biosynthesis and thus account for the antibiotic action. A more detailed understanding of this complex formation, however, is not possible without a knowledge of the molecular structure of the antibiotic.

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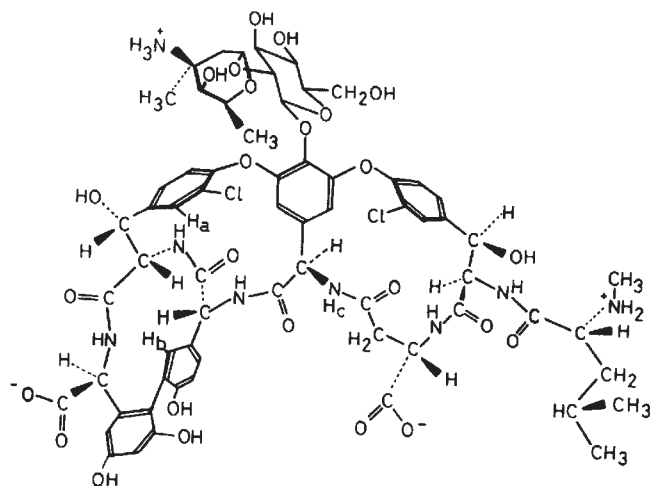


Fig. 1 The structure of CDP-I.

The structure of vancomycin has now been determined from an X-ray analysis of CDP-I⁶, a degradation product obtained from vancomycin with loss of ammonia. The solution of the structure, which contains 101 atoms (excluding hydrogens) in the asymmetric unit as well as 33 disordered water molecules, was obtained by direct methods, with the minimum use of chemical information. The analysis was based on full data out to a resolution of 0.8 Å, and without such extensive data it is unlikely that the structure could have been solved by the techniques available at present.

X-ray analysis of CDP-I confirms the structural units deduced from chemical, nuclear magnetic resonance (NMR), and mass spectrometry experiments⁶⁻¹². The principal units in the structure, summarised in Fig. 1, are a disaccharide linked to three aromatic rings; *N*-methylleucine; aspartic acid and a biphenyl system. These units are linked by secondary amide bonds, to form a tricyclic molecule, containing *N*-terminal *N*-methylleucine and two free carboxyl groups.

Aspects of the conformation of vancomycin in DMSO solution (from NMR data) correlate closely with the three-dimensional structure determined by X rays. There are thus no substantial conformational changes resulting from hydration or crystal forces. We present here details of the X-ray analysis, the three-dimensional structure and some suggestions concerning the mode of binding of acetyl-D-alanyl-D-alanine to vancomycin. If suitable crystals can be obtained, we hope to test these speculations by an X-ray analysis of such a complex.

Experimental details

Large crystals of CDP-I were obtained by dissolving vancomycin in water, adjusting the pH to 4.2 with a few drops of 0.05 N aqueous NaOH, and keeping the resulting solution at 70–80 °C for 40 h. During the course of the reaction, large, highly insoluble amber crystals separated out, one of which was cut to a rectangular block 0.7 × 0.3 × 0.2 mm and mounted in a capillary tube in contact with some mother liquor. Cell constants were measured on a 4-circle Syntex diffractometer using Cu Kα radiation (Table 1). The cell dimensions were slightly larger than previously reported¹³, probably because of increased hydration in this sample.

In total, 11,986 reflections, including Friedel pairs, were measured on the diffractometer in the range $0 < 2\theta < 101^\circ$ giving 7,759 unique reflections with $F > 3\sigma(F)$. A repeatedly monitored reference reflection decreased in intensity by ~20% during the 5 weeks of data collection and other intensities were scaled accordingly.

Solution of the structure

Numerous fruitless attempts were made to solve the structure using both Patterson and direct methods. Eventually a chemically sensible fragment, containing a six-membered ring and an atom

Table 1 Crystal data for CDP-I

Formula	C ₆₆ H ₇₆ Cl ₂ N ₈ O ₂₅ ·xH ₂ O
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>Z</i>	4
<i>a</i>	13.926(5) Å
<i>b</i>	21.578(5) Å
<i>c</i>	33.802(14) Å
<i>V</i>	10157(10) Å ³
Radiation	Cu Kα, λ = 1.5418 Å
μ	13 cm ⁻¹

assumed to be chlorine, emerged fortuitously from one of the E-maps. Tangent expansion and guesswork extended this to the three aromatic rings of the central nucleus and the two chlorine atoms. All attempts to expand this fragment, including the use of translation functions, were unsuccessful, until the geometry had been optimised by least-squares refinement with distance constraints. A single tangent expansion then produced 80 atoms, all of which could be identified with the units deduced chemically. Further atoms, including the water molecules, were located from difference syntheses.

The structure has now been refined to a current *R* of 16%. All atoms except Cl are isotropic. Fifty-four H atoms were included at calculated positions (C–H 1.08 Å, H–C–H 109.5°); 33 water molecules were located, with site occupancy factors varying from 0.47 to 1. The temperature factor for the disordered water molecules was fixed at 0.15 Å². Refinement is continuing. The absolute configuration has not been determined from the X-ray data, but it must be that shown in Fig. 2 from the known stereochemistry of chemically isolated fragments.

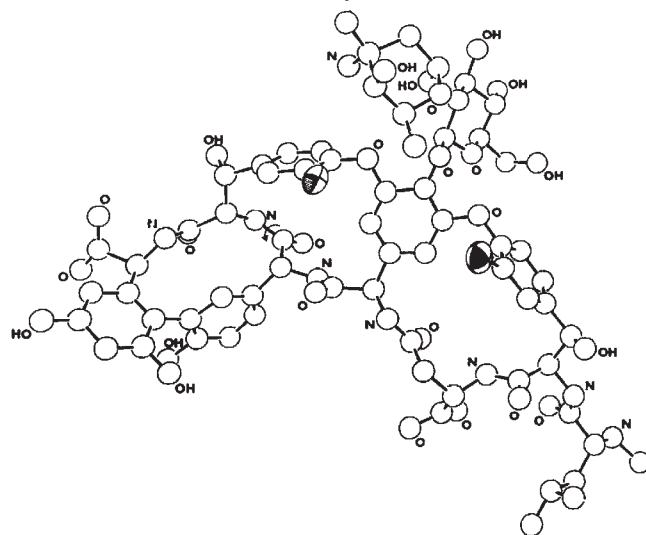
The structure

As already mentioned, all the units deduced from chemical and spectroscopic evidence have been confirmed by the X-ray structure. These include the amino sugar vancosamine, two β-hydroxychlorotyrosine units and three oxygenated phenylglycine systems as well as *N*-methylleucine and aspartic acid. It is unusual that in the latter, the α carbonyl is present as the free carboxyl group. The structure also contains a relatively rare *cis* amide bond.

The molecule is extremely compact, as can be seen from the space filling drawing of Fig. 3. It carries a marked cleft on the side of the molecule bearing the Cl atoms, but also a less well-defined cleft on the other side. There is no space for any water molecules inside the tricyclic ring system.

The molecules are linked in the extended crystal structure by a complex system of hydrogen bonds. There are only five hydrogen

Fig. 2 ORTEP¹⁶ plot of CDP-I. The *cis* amide bond is arrowed; H atoms are omitted. Anisotropic atoms are chlorine.



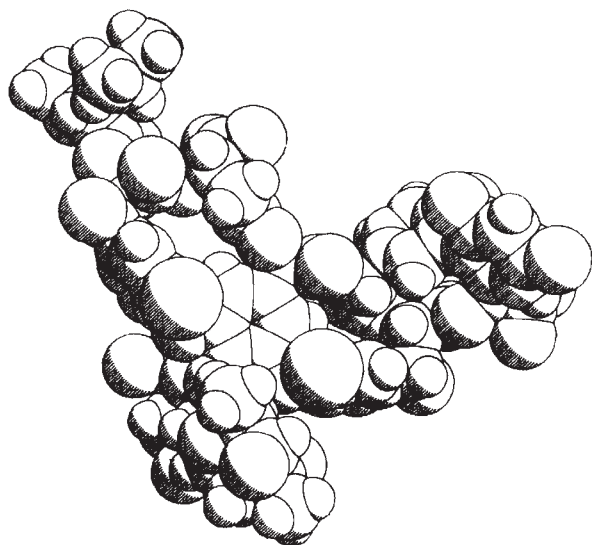


Fig. 3 Space-filling plot of CDP-I (same orientation as Fig. 2, using the program PLUTO, W. D. S. Motherwell, unpublished). The following radii were used (Å): C, N, O all 1.4; Cl 1.7; H 0.9. Only H atoms attached to C are included.

bonds which do not involve water: the carboxyl of aspartic acid to OH of the trisubstituted ring of the biphenyl, NH_2 of methyl-leucine to carboxylate of the biphenyl, O4 of the glucose to NH of the diaryloxyphenyl-glycine and O4 of vancosamine to the carboxylate and NH of aspartic acid. The remaining hydrogen bonds are between the CDP-I molecule and water molecules, and between the water molecules themselves, forming an intricate three-dimensional network.

CDP-I is obtained from vancomycin by the loss of ammonia. The free carboxyl group on the biphenyl group of CDP-I is known also to occur in vancomycin. The structure of vancomycin is therefore that shown in Figs 1 and 2 except that the carboxyl group of the aspartic acid is changed to a primary amide ($-\text{CONH}_2$).

Mode of binding

The molecular basis of the antibiotic action is probably associated with the binding of acyl-D-Ala-D-Ala fragments into the cleft of the molecule on the face bearing the chlorine atoms. Earlier NMR studies^{14,15} have shown that the C-terminal alanine methyl resonance of Ac-D-Ala-D-Ala is heavily shielded in the presence of vancomycin. Later work¹² established that binding of Ac-D-Ala-D-Ala to vancomycin caused marked downfield shifts of protons

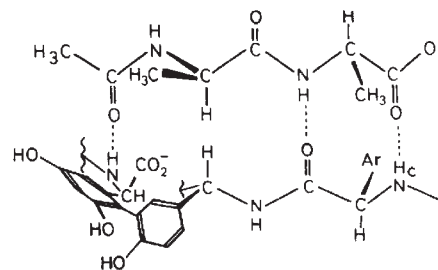


Fig. 4 Schematic illustration of a possible interaction between Ac-D-Ala-D-Ala and a portion of the vancomycin molecule.

H_b and H_c , and a marked upfield shift of H_a (Fig. 1). It is likely that these protons are near to the peptide fragment in the antibiotic-peptide complex, and in particular that NH_c is hydrogen bonded to a peptide carbonyl group. On the basis of this evidence, and an examination of space-filling models, we propose that vancomycin associates with the backbone of Ac-D-Ala-D-Ala as shown in Fig. 4, where the aromatic residue (Ar) is the trioxxygenated benzene ring bearing the disaccharide. In the proposed complex, the C-terminal methyl group of Ac-D-Ala-D-Ala lies above the face of this benzene ring, thus accounting for its upfield shift on complex formation. The other secondary methyl group of Ac-D-Ala-D-Ala lies near the benzene ring which carries two phenolic hydroxyl groups, but is not located centrally over it and so is not similarly shielded on complex formation.

This model, although consistent with the X-ray structure and spectroscopic results, is not necessarily a unique one, and further experimental evidence is needed to establish the precise mode of binding of the antibiotic-peptide complex.

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letters to nature

Positions of galactic X-ray sources: $320^\circ < l^{\text{II}} < 340^\circ$

PRECISE ($20-40''$) positions of seven X-ray sources in the celestial region $320^\circ < l^{\text{II}} < 340^\circ$ are reported here. These include a recurrent transient X-ray source (MX1608-52; refs 1-4) and a source 2S1553-542 (ref. 5) coincident with a γ -ray source⁶ within the given errors of position. The positions reported here reduce the previously reported areas

of the error region for six of the sources by factors ranging between 10 and 100. In the case of MX1608-52, a preliminary report of this work² led to the identification of an optical candidate^{7,8}. The present results add confidence to the proposed radio candidates for 4U1624-49 (ref. 9) and 4U1642-45 (refs 9, 10). But they eliminate proposed possible optical candidates^{5,11-14} for the sources 4U1543-62, 2S1553-542, 4U1624-49 and 4U1642-45.

The positions for the sources 4U1538-52, 4U1543-62, 2S1553-542, (MX1553-54), 4U1556-60, MX1608-52,

Table 1 Celestial positions

SAS-3 designation	Other designations*	Position (1950) α	δ	l^{II} b^{II}	Error radius (90%)	Flux density† (2–11 keV)	Comments
2S1538–522	4U1538–52	15h38m40.2s 234.6675°	–52°13'38"– –52.2272°	327.42° 2.16°	40"	9 μ Jy	Pulsing binary ^{23, 25, 26}
2S1543–624	4U1543–62	15 43 33.9 235.8912	–62 24 56 –62.4155	321.76 –6.34	30	38	
2S1553–542	MX1553–54	15 53 55.6 238.4817	–54 16 15 –54.2708	327.95 –0.86	35	27	γ -ray source CG327–0? (ref. 6)
2S1556–605	4U1556–60	15 56 47.2 239.1967	–60 35 47 –60.5963	324.15 5.93	30	16	
2S1608–523	MX1608–52 4U1608–52	16 08 51.2 242.2133	–52 18 02 –52.3005	330.93 –0.85	20	618 $\pm$	Recurrent transient ^{2–4} Burst source ^{7, 30, 31} Optical candidate ^{7, 8} Radio candidate ⁹
2S1624–490	4U1624–49	16 24 19.6 246.0817	–49 05 07 –49.0853	334.92 –0.26	35	49	
2S1642–455	4U1642–45 GX340+0	16 42 09.9 250.5412	–45 31 10 –45.5194	339.59 –0.08	30	338	Radio candidate ^{9, 10}

*Refs 1, 5, 20, 32.

†1.0 μ Jy corresponds to 2.2×10^{-11} erg s^{–1} cm^{–2} (2–11 keV). $I_{\text{crab}} = 1,060$ μ Jy (see refs 15, 16).

‡At the time of observation (24 July, 1977).

4U1624–49, and GX340+0 (4U1642–45) are reported in Table 1 along with the estimated error radii¹⁵. The observations of six of these sources were carried out with the SAS-3 rotation modulation collimator from 16 June to 21 June 1975 as part of a survey of galactic source positions^{15–19}. The source MX1608–52 was observed on 24 July 1977 when it was in a bright state². In Fig. 1, we compare our results with other measurements and positions of radio and optical candidates for three of the sources. Finding charts for six sources are given in Fig. 2. Astrometry and photometry results for a few stars in the error regions are given in Table 2.

Ten other known X-ray sources lie in this region. SAS-3 positions for three of these (Cir X–1, TrA X–1 = A1524–61, MXB1636–53) were reported earlier^{17, 18}. The remaining seven were not seen in our survey. Of these, the sources 4U1543–47 and 4U1630–47 are known transients^{20, 21} and the source 4U1658–48 is known to be highly variable²². We place an upper limit of 10 μ Jy (2–11 keV) for these and the other four sources (4U1510–59, 4U1530–44, A1540–53 and 4U1631–64). The intensity of MX1608–52

was ≤ 5 μ Jy during the 1975 observations. These limits are consistent with the intensities and variability factors previously reported for these sources^{20, 22}.

We discuss briefly below the proposed radio and optical candidates for the sources:

4U1538–52: Cowley *et al.*²⁴ have measured the colours for ~ 50 stars in the Uhuru error region and have suggested several probable candidates for this source; stars numbered 10 and 12 in Fig. 2 are among these. This source is now known to be the same as A1540–53 (ref. 23), on the basis of new Ariel V position determinations²⁵ and is also known to be a pulsing binary system^{23, 25, 26}.

4U1543–62: The star suggested as a possible candidate by Penston *et al.*¹¹ (denoted as A in their Plate II) is excluded as a candidate.

2S1553–542: The variable star TT Normae and the B0 supergiant SAO243166 are near our error circle (Fig. 1). We previously suggested³ the latter as a candidate. Subsequently, failure to detect emission lines or velocity variations in its spectrum²⁷ weakened the proposed identification. This star is $\sim 1.2'$ away from the refined position given here and

Fig. 1 X-ray source positions and candidates for three sources. The SAS-3 error circles for the other sources are smaller in area than previous work by factors of 10–100. The positions are from Uhuru (U, ref. 20), and Ariel V (A, ref. 33) satellites, a rocket experiment³⁴, and the present work (S). The stars TT Normae and SAO243166 near 2S1553–542 and the star SAO226781 near 2S1624–490 are also shown. The position of the radio candidate⁹ for 4U1624–49 and the extent of a finite-sized radio source^{9, 10} coincident with 4U1642–45 are indicated.

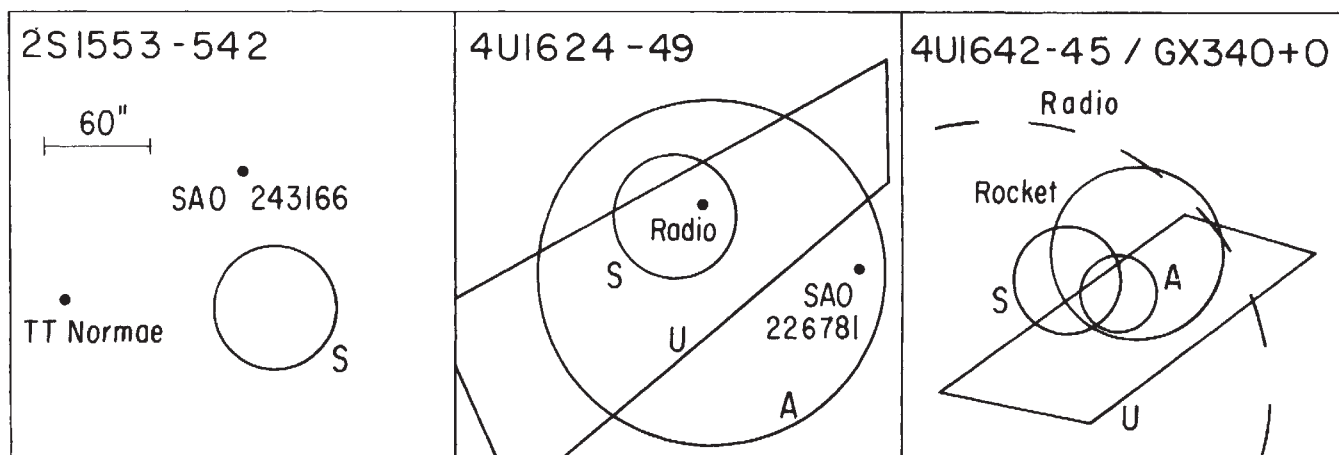


Figure 1

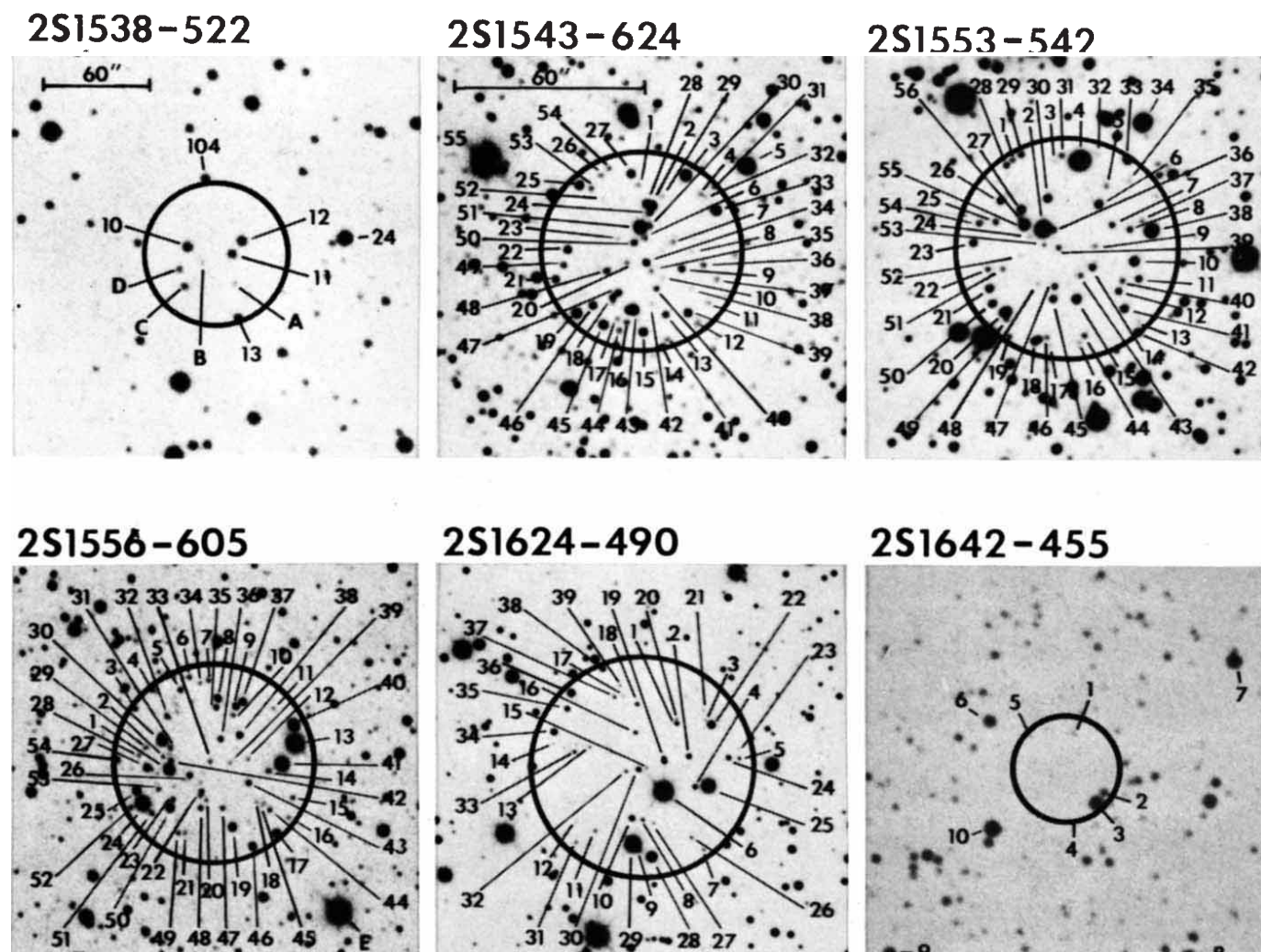


Fig. 2 Finding charts for six SAS-3 positions. A finding chart for 2S1608-523 is given by Grindley and Liller⁸. The charts for 2S1553-542 and 2S1624-490 are from R plates and those for 2S1543-624 and 2S1556-605 from B plates taken with the CTIO telescope by W. A. Hiltner and C. Canizares; see chart 2S1543-624 for scale. The other two charts are from the FSO quick-blue survey; see 2S1538-522 for scale. We adopted the numbering scheme of ref. 24 for 2S1538-522 and the lettering scheme of ref. 11 for 2S1556-605. North is up and east is to the left.

is excluded as a possible candidate. This source is within the 1° radius error circle of the γ -ray source CG327-0 discovered by the COS-B satellite⁶.

4U1556-60: The star¹⁴ SAO253382 excluded as a candidate by the Copernicus satellite measurement^{11,28}, is also excluded by the present observation.

MX1608-52: The star SAO243445 within the Uhuru error box²⁹ (denoted as star A in Fig. 1 of ref. 29) is excluded by the present measurement. Grindley^{7,8} reports a candidate star within our error circle, which brightened from $V > 23$ mag in August 1976 to $V = 21 \pm 1$ mag in August 1977. The present position is within the error region of the Norma X-ray burst source^{30,31}.

4U1624-49: The radio source G334.9-0.3 proposed by Sanduleak and Dolan⁹ as a candidate is supported by the present measurement (Fig. 1). The star SAO226781 considered as a possible candidate¹¹⁻¹⁴ is excluded by the present observation.

4U1642-45: The extended radio source G339.6-0.1 proposed by Sanduleak and Dolan⁹ and by Seaquist¹⁰ as a candidate is supported by the present observation (Fig. 1). Seaquist¹⁰, on the basis of the flat radio spectrum, suggests that the radio source is an HII region. The star suggested as a possible candidate by Penston *et al.*¹¹ (denoted as A in their plate III) is excluded by the present measurement.

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Table 2 Stellar astrometry and magnitudes

Source	Star	m_v^*	Position (1950) [†]	
			α	δ
2S1538-522	24	13.7 $\ddagger$	15h 38m 32.3s	-52 13' 34.8"
	5		15 43 29.1	- 62 24 27.1
2S1543-624	3	15.3		
	55		15 43 41.2	- 62 24 25.1
2S1553-542	34		15 53 52.8	-54 15 30.8
	2	15.0		
2S1556-605	4	13.8		
	E	11.9 $\S$	15 56 42.0	60 36 32.2
2S1624-490	13	13.3		
	6	12.6	16 24 18.7	-49 05 13.8
2S1642-455	28	15.9		
	3		16 42 08.2	45 31 27.7
	6		16 42 13.9	-45 30 42.7

*From photoelectric photometry by J. E. McClintock accurate to 0.1 mag.

[†]Precise to $\leq 3''$.

[‡]From ref. 24.

[§]From ref. 11.

Aeronautics and Space Administration. This is the sixth in a series of articles on positions of X-ray sources obtained with SAS-3.

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Dynamic measurement of matter creation

If a rotor could be spun so that it had an inertial decay time $\sim 10^{11}$ yr, 10^{18} s, it could test matter creation cosmologies¹⁻³ at a significant level. The advantage of this method over previous ones⁴⁻⁸ is that the result is independent of the form in which matter is created, providing it stays in place. We have designed an experiment to test this process; it is under construction and is described here. It consists of an inertially spinning rotor suspended inside another rotor, a modification of the double magnetic suspension⁸. Past measurements or estimates have not unequivocally answered the question of matter creation. Cohen and King⁴ established an upper limit of 4×10^{-23} g per g per s on the creation rate of hydrogen in mercury metal. Gittus⁵ suggested that matter created in rocks takes the form of interstitial atoms which diffuse into dislocations. Towe⁶ disputed this, noting that if mass proportional creation were occurring, 30% of the atoms in old terrestrial rocks would occupy interstitial positions and this would result in lattice disruptions. Gittus⁷ later proposed that the matter multiplication rate for crystalline materials could be found by determining the ratio of the shear modulus to the crystal viscosity. He suggested that a reasonable estimate for the sensitivity of the method might be 3×10^{-18} atoms per atom per s, although this has not been tested. In a geophysical argument, Wesson⁸ can account for

the observed expansion rate of the earth by appealing to a mass proportional matter creation whose rate is 7×10^{-18} g per g per s, but he notes that this is in conflict with the result of Cohen and King; it exceeds Dirac's prediction by several times.

Figure 1 shows the elements of the experiment. Two cylinders of highly temperature-stable ceramic rotate concentrically about the axis of rotation of a precision turntable. The outer cylinder is connected to the turntable which rotates with an integral speed constancy limited only by the precision of the atomic clock. The turntable drive is a 50-pole magnetic-averaging synchronous direct drive and the sensor is a double-plate totally averaging encoder system.

The inner (inertial) cylinder is magnetically suspended from the outer and initially caused to rotate with the outer, after which it is freely rotating. Mass created in the inner cylinder causes it to slow down relative to the outer cylinder. The accumulated phase lag of the inertial cylinder provides an extremely sensitive measure of this relative motion. A synchronised pulsed laser has a split beam, part of which is for sensing the relative angle of the two cylinders and another part of which, when unblocked, provides forward or backward momentum impulses to the inertial cylinder to keep it in phase. The sequences of the pulses constitutes the signal. Before and after experiments calibrate the momentum impulses and also provide estimates of limits on competing energy loss mechanisms when the inertial cylinder is driven up to speed from rest with the turntable stationary.

Three major competing mechanisms for energy loss among among many^{9,10} are (1) viscous losses in the residual gas of the vacuum, (2) magnetic hysteresis in the support, and (3) interaction with external magnetic fields. Another consideration is the change in cylinder radius due to thermal expansion, and a further major factor is the wandering of the inertial cylinder about its equilibrium position due to thermal noise.

The viscous losses and magnetic hysteresis are rendered virtually zero by the feedback system described above which allows essentially no relative motion between the cylinders. Shields can reduce asymmetric (hysteresis) effects of external magnetic fields to insignificant levels. Thermal expansion effects are kept negligible if the system is thermally isolated and allowed to come to temperature equilibrium, $\Delta T \leq 10^{-3}$ K and by making the cylinder of a material with temperature coefficient $\alpha < 10^{-7}$ K⁻¹. In this case $\Delta r/r < 10^{-10}$ and constitutes a short term variation only, not a steady drift.

The thermal fluctuations can be estimated from standard noise criteria:¹¹

$$\frac{1}{2} I (\Delta \omega_n)^2 = -\frac{1}{2} kT, \quad (1)$$

where I is the inner cylinder moment of inertia, kT is the thermal energy and $\Delta \omega_n$ is the thermally caused fluctuation in velocity.

Then if

$$\Delta \theta_n = \int \Delta \omega_n dt, \quad (2)$$

is the angle through which the cylinder has random-walked from synchronous position due to noise, we get

$$\Delta \theta_n = (kT/I)^{1/2} \tau, \quad (3)$$

where τ is the duration of the experiment.

The magnitude of the expected change in angle due to mass creation depends on the particular cosmology by which it is predicted. If the newly created matter has the same velocity as the matter in the inertial cylinder, then no effect might be measured even if mass creation existed. But as the newly created matter presumably owes its existence in some way to the Universe as a whole, we might expect its initial state to reflect the overall rotation of the Universe which we presume to be zero. In this case, there would be some 'drag' on the inner cylinder.

There are still many possibilities, three of which we will mention. First, angular momentum, L , is conserved and mass

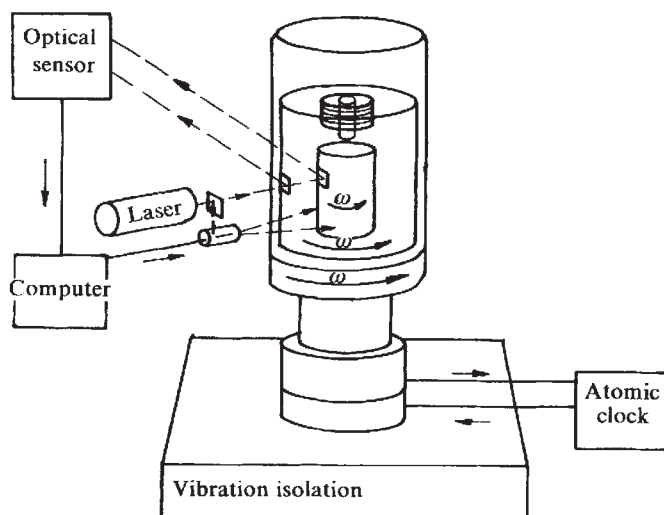


Fig. 1 Two cylinders of temperature-stable ceramic (Zerodur) rotate concentrically in an evacuated region inside an acoustic and magnetic shield. The inner cylinder is magnetically suspended from the outer one which rotates with precise angular velocity ω . Mass created in the inner cylinder tends to slow it down. A feedback system employing laser pulse sensing and photon driving keeps the inner cylinder velocity ω' very near to ω . Forward-backward asymmetry needed in these feedback driving pulses to keep $\omega' = \omega$ constitutes the signal. With the two cylinders running synchronously, viscous, magnetic hysteresis and other damping effects are kept near zero.

is created with no angular momentum and in such a way that the dimensions of the cylinder remain constant. If $I =$ moment of inertia, $M =$ mass, and $r =$ radius of the cylinder, then $L = \text{constant} = I\omega \sim Mr^2\omega$ which gives $\dot{\omega}/\omega = -\dot{M}/M$. Second, L conserved; mass created with no angular momentum and density is conserved. As the arguments are dimensional, we will treat the height of the cylinder as the radius, then $L = \text{constant} = \rho^{-2/3} M^{5/3} \omega$ which gives $\dot{\omega}/\omega = -5/3 \dot{M}/M$.

Third, large numbers hypothesis as for instance in ref. 1. Under the LNH angular momentum is not conserved in atomic units. Velocity and atomic quantities are constant. If the new matter is created such that density, which is an 'atomic' property is constant, then $\omega \sim v/r \sim v(\rho/M)^{1/3}$, or $\dot{\omega}/\omega = -1/3 \dot{M}/M$. (It is possible to analyse a rotor in more than one way under the LNH; for example, the spinning earth is gravitationally bound and, hence, its density is not purely an atomic property. In this case the analysis might proceed more along the lines of an orbit problem and one gets $\dot{\omega}/\omega \sim -1/2 \dot{M}/M$).

In all of these cases we find $\dot{\omega}/\omega = -Y\dot{M}/M$ where Y is a number of order unity. Typical estimates for $\dot{M}/M$ are $2 \times 10^{-10} \text{ yr}^{-1} = 6 \times 10^{-18} \text{ s}^{-1}$. If the inner cylinder has no initial motion relative to the outer, then after a time τ we would have an angular displacement

$$\delta\theta = \omega\tau^2 = -Y\dot{M}/M \tau^2. \quad (4)$$

Thus, the lag angle increases quadratically in time. Equations (3) and (4) can be combined to give the signal-to-noise ratio $\delta\theta/\Delta\theta_n$, from which the sensitivity to mass creation is

$$\dot{M}/M = [(\delta\theta/\Delta\theta_n) (kT/I)]^{1/2} / Y\omega\tau \quad (5)$$

Without feedback, the sensitivity from equation (5) at room temperature, with $I = 25,000 \text{ g cm}^2$ and $\omega = 10 \text{ rad s}^{-1}$ would be $\dot{M}/M = 10^{-8}$. Feedback if noiseless and of gain G can reduce the effective temperature¹¹ by a factor of $\sim (G)^{1/2}$, and the experiment ultimately will be cooled as well. Appropriate feedback will be controlled by a digital processor. Thus the signal-to-noise condition can be met simultaneously with the

low damping requirement. To emphasise the level of difficulty of the experiment we note, from the secular decrease in the earth's rotational angular velocity¹², largely due to tidal friction, that the earth itself as a rotor is too dissipative by a factor of 20. Braginsky¹³ has shown from noise considerations and fundamental limits on detection sensitivity that mechanical oscillators can in principle have decay times $\sim 10^{-18} \text{ s}$. Similar limits apply to rotors and estimates with our parameters are that this can even be improved.

Ultimately, an observed effect would always be suspected of unaccounted-for-damping, yet most damping effects are expected to be linear in time to a close approximation and the signal is quadratic so that it has a distinctive signature. Nevertheless, if an effect is observed the experiment will be repeated at different initial angles, with different cylinders, and at different temperatures.

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Identification of interstellar polysaccharides and related hydrocarbons

INFRARED transmittance spectra of several polysaccharides which are suitably calibrated for comparison with astronomical data have been obtained. We show here that a mean 2.5-15 μm spectrum computed from our measurements is remarkably close to that required to explain a wide range of astronomical data, except for two significant points of departure. The required relative opacity at the 3 μm absorption dip is a factor ~ 1.5 lower than we found in our laboratory measurements: this difference may arise from the presence of water ($\sim 10\%$) associated with terrestrial polysaccharide samples. In the 9.5-12 μm waveband an additional source of opacity seems to be necessary. The close agreement between the spectrum of this excess opacity and the absorption spectrum of propene C_3H_6 points strongly to the identification of hydrocarbons of this type which may be associated with polysaccharide grains in interstellar space.

The presence of interstellar polysaccharides has been deduced¹ by comparing infrared spectra of several galactic infrared sources in the 2-30 μm waveband with model calculations based on transmittance data for cellulose. We show here that the transmittance properties of cotton cellulose gives a good agreement to the observed emission from the Trapezium nebula in the 8-30 μm waveband. But with the adoption of more stringent standards of comparison between observations and theory, we thought it appropriate to 'optimise' the transmittance values over the waveband 2.1-13 μm without departing from the original cotton cellulose data by more than a reasonable margin. In the absence of properly calibrated transmittance data for other polysaccharides we have provisionally assigned our optimal values to a 'notional' interstellar polysaccharide ensemble. It is, therefore, desirable to obtain

experimentally a mean transmittance curve for several related polysaccharides.

Four polysaccharides: α -cellulose (α -cellulose fibre ~99.5% pure), amylose (from potato), arabinogalactan (from Larch wood) and mannan (from bakers yeast) were included in the present study. Samples supplied by Sigma London Chemical Company Limited were dispersed in KBr disks at concentrations

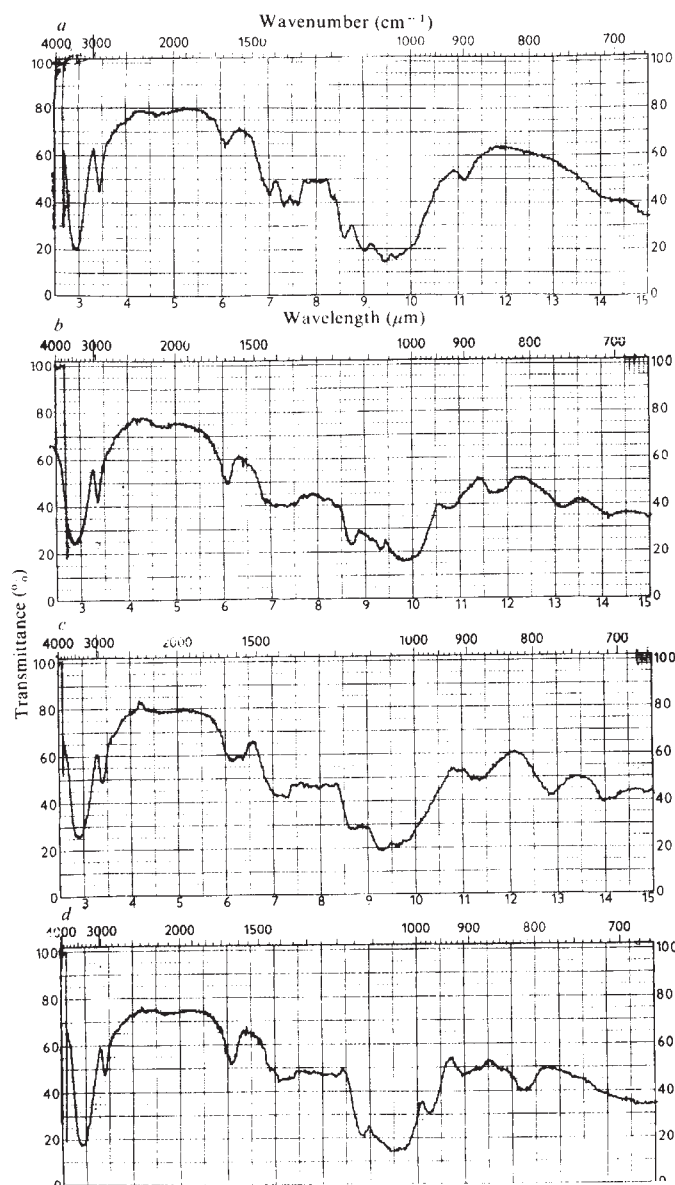


Fig. 1 Transmittance spectra of several polysaccharides dispersed in KBr. *a*, α -cellulose; *b*, amylose; *c*, arabinogalactan; and *d*, mannan. Concentrations are about 1% with respect to KBr. Spectra shown here were obtained using a Perkin-Elmer 137 Infracord Spectrophotometer.

of about 2 mg per 200 mg KBr, and spectra obtained using either a Perkin-Elmer 137 Infracord spectrophotometer or a Perkin-Elmer 257 Infrared spectrophotometer. The spectrophotometer was adjusted to give a 100% transmittance reading at all wavelengths in the absence of the mounted disks. Thus the measured transmittance (%) corresponds to $100 \exp(-\tau_\lambda)$ where τ_λ is the opacity of the laboratory samples. All these spectra (Fig. 1) show the characteristic signatures of polysaccharides, with principal absorptions centred on 3, 3.4, 6 and 9.5 μm . The solid curve in Fig. 2 shows the mean transmittance curve $\exp(-\langle\tau\rangle)$ computed from the spectra in Fig. 1.

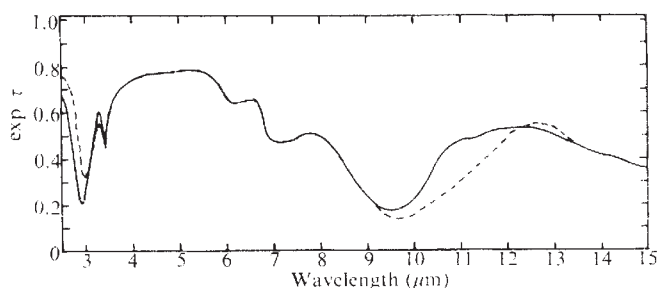


Fig. 2 Mean transmittance spectrum for four samples from Fig. 1. Dashed segments represent optimised variations to give best fit to astronomical spectra.

The agreement of this curve with the spectrum of α -cellulose is close, so our earlier attempts to use a smoothed-out cellulose transmittance curve to represent the properties of interstellar polysaccharides would seem to be justified.

We shall now use our mean polysaccharide transmittance curve to interpret spectra of galactic infrared sources. Using our cascade model for the formation of polysaccharides in mass flows from stars^{2,7} infrared spectra were computed from several sets of transmittance data. Although tolerable fits to astronomical spectra are obtainable with any one of the transmittance curves in Fig. 1 or with the mean curve (solid line) in Fig. 2, the requirement for an excellent fit to a wide range of observations demands minor modifications to our mean polysaccharide transmittance data. These modifications are indicated by the dashed lines in Fig. 2. Figure 3 shows the detailed agreement which is obtained using this modified transmittance data in a source calculation for the BN object. The excellent agreement between observations and theory gives strong empirical support for the modifications proposed here. But even these apparently minor departures from our polysaccharide mean data need interpretation.

The general similarity of the empirically estimated optimal transmittance data to the mean polysaccharide curve is very close except in two respects. (1) The value of $\tau(3\mu)$ for the experimental samples (and the mean curve) are a factor ~1.5 higher than what we infer from astronomical observations. We are most probably seeing here the effect of significant amounts of H_2O associated with freeze-dried terrestrial polysaccharides. Astronomical polysaccharides will be largely free of water, and the 3 μm absorption significantly shallower. A factor ~1.5 could well arise due to this effect. We note that a

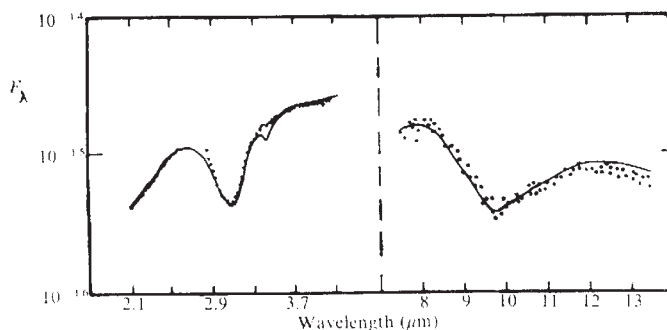


Fig. 3 Solid curve is infrared emission for the source BN calculated for the model of Hoyle *et al.*⁷. The formation temperature of polysaccharides was taken as 850 K and the grain temperature was taken to vary as the inverse square root of the distance from the exciting star. The optical depth of the region of formation of the grains was four times that of the mean sample data of Fig. 2. The points represent the observational data of Gillett and Forrest⁸ and of Merrill *et al.*⁹.

reduction in $3\text{ }\mu\text{m}$ opacity by a factor ~ 1.3 already exists between native cotton cellulose and mercerised cotton studied by O'Connor³.

Astronomical observations also show minor shifts in the central wavelength of the $3\text{ }\mu\text{m}$ absorption dip with transmittance minima in the range $2.9\text{--}3.05\text{ }\mu\text{m}$ compared with minima in the range $2.8\text{--}2.9\text{ }\mu\text{m}$ for the laboratory systems we have considered. Wavelength shifts of this magnitude could arise for interstellar polysaccharides due to several effects: variations in the extent of hydrogen bonding between chains, particularly if individual chains are separable; effects of attached side

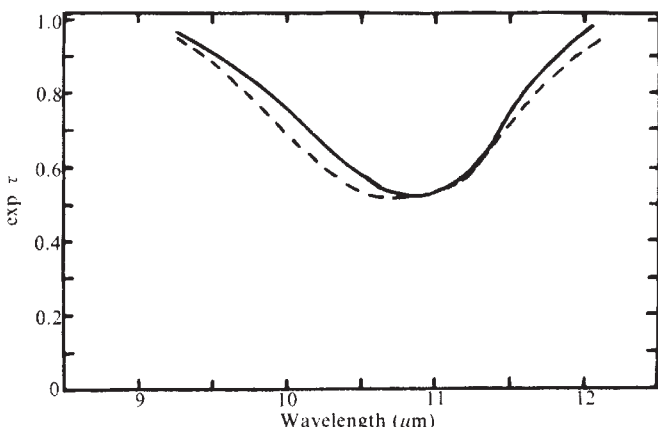


Fig. 4 Required excess opacity (transmittance) in $9\text{--}12.5\text{ }\mu\text{m}$ waveband compared with a normalised spectrum of C_3H_6 (propene). The normalisation involves multiplication of the opacities τ in the two cases by factors which equalises the $11\text{ }\mu\text{m}$ transmittance values in the two cases. Observed $\exp(-\Delta\tau)$ (---); normalised spectrum of C_3H_6 (—).

groups in the polysaccharides; and particle size effects if grains grow to sizes larger than 10^{-6} cm .

(2) The astronomical data require an additional source of opacity in the $9\text{--}12.5\text{ }\mu\text{m}$ waveband which must be superimposed upon the polysaccharide transmittance spectrum. This is precisely the waveband where many carbon stars show an excess emission^{4,5} peaking at $\sim 11\text{ }\mu\text{m}$ which is readily explained by the presence of a mixture of hydrocarbons. The excess transmittance $\exp(-\alpha\Delta\tau)$ for our dashed curve in Fig. 2 is shown in Fig. 4. The constant α is chosen appropriately for comparison with a suitably normalised laboratory transmittance spectrum⁶ for propene (C_3H_6). The normalisation arbitrarily equalises the value of the transmittance at $11\text{ }\mu\text{m}$ in both cases. The remarkably close agreement leads us to infer the presence of a similar material which is apparently closely associated with interstellar polysaccharides. Hydrocarbons of this type may be produced as an intermediary in the building process of polysaccharide chains in sources, and also as a result of a partial degradation (similar to a coalification process) of polysaccharides in interstellar space.

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Change in nature of stratospheric aerosol collected at 34°S

AEROSOL collected from the stratosphere at Mildura (34.2°S , 142.1°E) has undergone a marked change in character after a period of about seven years with little variation. During the period 1970 to February 1977 aerosol collected by jet impaction at altitudes up to about 28 km has predominantly been sulphuric acid, except for a brief period in January 1972 when particles with a wide variation in morphology were observed¹. The change reported here indicates a substantial incursion of ammonia into the stratosphere, sufficient to almost completely convert the sulphuric acid to one of the ammonium salts, either NH_4HSO_4 or $(\text{NH}_4)_2\text{SO}_4$.

Increased ammoniation is indicated principally by the change in morphology of particles collected (during ascent) on a carbon surface and 'shadowed' with silicon oxide later in the laboratory. The sequence is shown in the T.E.M. photographs in Fig. 1*a*, *b* and *c*. The flights are: *a*, fl.667, 2 February 1977; *b*, fl.672, 28 April 1977; *c*, fl.675, 1 July 1977. The particles shown in Fig. 1 were all collected near 18 km altitude but are in fact representative of the overall aerosol layer between the altitudes of about 16 and 28 km . Particles from flight 667 consist of a small central spherical cap surrounded by rings of acid droplets. This is typical of the aerosol collected over the past seven years and also of aerosols generated from H_2SO_4 in the laboratory and indicates a large proportion of free sulphuric acid in these aerosols. On the April flight, the relative increase in size of the central particles and their 'eroded' appearance points to an increase in ammonia content, while particles collected on the July flight (Fig. 1*c*) clearly show the morphology of ammonium sulphate with only a very small acid component, that is a flattish eroded dome with a few surrounding droplets. Many of the particles collected at a higher altitude on this flight were found on examination to be in a crystalline form, recognisable as $(\text{NH}_4)_2\text{SO}_4$.

The changed appearance of etch marks left by particles impacted on a copper surface and then subjected to a humid atmosphere is another indication of the changed nature of the aerosol, although this change is non-specific.

Clear etch marks typical of sulphuric acid are evident only on flight 667 (Fig. 1*d*) whereas the later flights, particularly 672, show a 'splashed' appearance, suggesting a moist or semi-liquid reactive aerosol (Fig. 1*e* and *f*).

Chemical testing by thin film techniques with BaCl_2 (refs 2, 3) a specific test for sulphate, shows that for all three flights sulphate is present as a major component in all particles within the radius range of approximately 0.03 to $1\text{ }\mu\text{m}$ (see Fig. 1*g*, *h* and *i*).

Neither the latitude of the NH_3 injection nor the mechanism of its transport into the stratosphere is known. If, however, we use mass loadings calculated from a flight that closely preceded the change a rough estimate of the quantity of NH_3 required to neutralise the H_2SO_4 present can be made. The concentration of particles observed by *in situ* photoelectric counting of individual particles on the flight of 12 November 1976 (J. E. Laby, unpublished) and the size distribution derived from our impaction studies yield a mass of $1.1 \times 10^{-10}\text{ kg}$ of H_2SO_4 in a 1 cm^2 column between the tropopause at 12.9 and 30 km . To neutralise this fully to $(\text{NH}_4)_2\text{SO}_4$ requires $3.7 \times 10^{-11}\text{ kg}$ of NH_3 , equivalent to a uniform mass mixing ratio of 2.3×10^{-10} . Distributed uniformly over the hemisphere the total mass involved would be a prodigious 10^8 kg . We may, therefore, assume until observations from other latitudes are available that the phenomenon was relatively local. Nevertheless, a very large total mass of ammonia

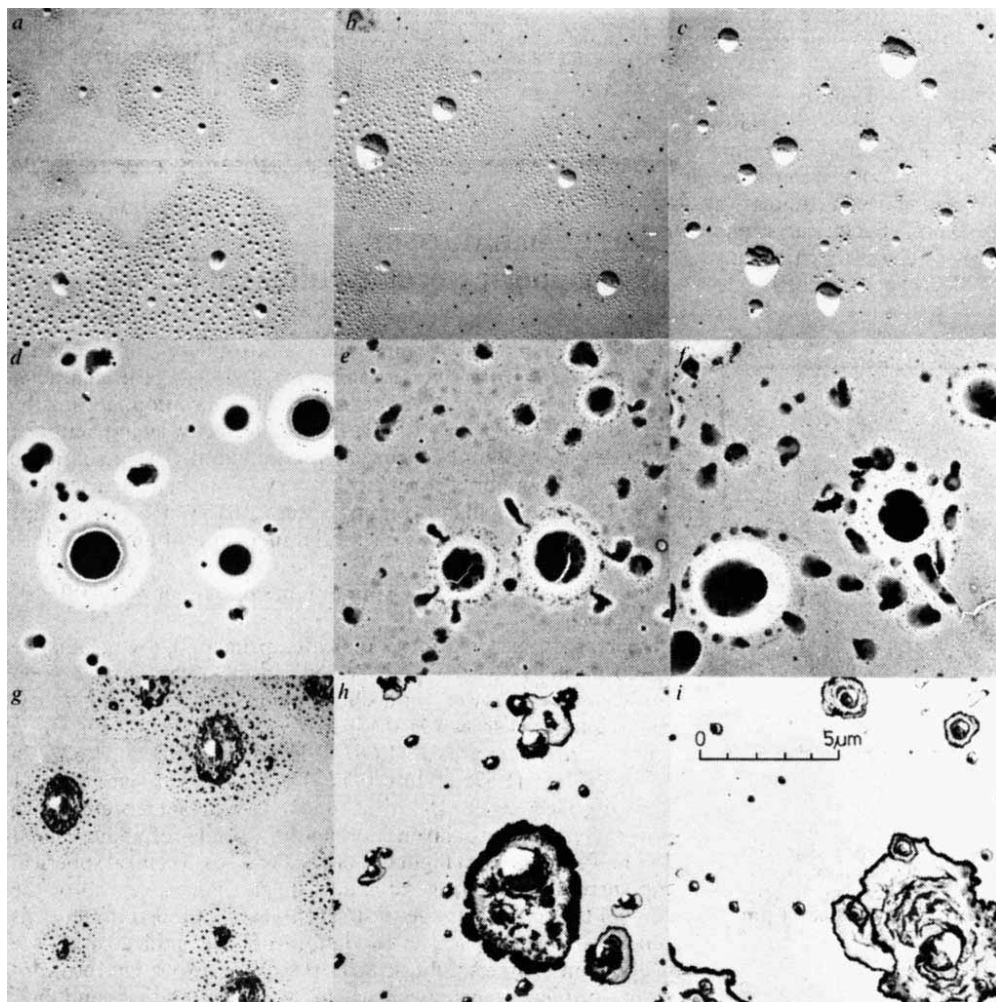


Fig. 1 Particles collected by impactation at approximately 18 km on *a*, 2 February; *b*, 28 April and *c*, 1 July 1977. Surface morphology is shown in the top row which shows particles caught on carbon surfaces and later 'shadowed'. A clear change from H_2SO_4 on the left to $(\text{NH}_4)_2\text{SO}_4$ on the right can be seen. The reactions of particles with a copper collection surface are shown in the centre row and in the bottom row the insoluble BaSO_4 precipitate rings which form after post-coating collected particles with a thin film of BaCl_2 and humidification indicate the presence of sulphate ion.

must have been involved, and it was certainly the most significant injection of the last seven years.

The observations reported here also reveal a phenomenon of particular relevance to stratospheric monitoring: an almost complete change in the nature of the aerosol which simply could not be determined with present remote optical sensing techniques. Direct collection of particles and examination by electron microscopy must remain an essential method of interpretation of stratospheric particulate sources and processes.

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Predicting concentrations in plumes subject to dry deposition

THE source depletion¹ estimation of dry deposition from the atmosphere is less accurate than the physically more correct surface depletion method^{2,3}. Several surface depletion models^{2–9} have been developed but the source depletion method is still used^{1,10}. The errors introduced by using the latter might have important consequences for assessing the impact of deposition from anthropogen sources. Only the recent^{4–6} studies discussed here quantify the discrepancy between the two deposition

methods. We suggest that a ratio R , based on a representative measure of the eddy diffusivity, plume height, and the deposition velocity, should be used to discriminate between cases where the source depletion method introduces significant errors and those where the difference between the two methods is negligible.

Vertical dispersion and dry deposition of material from low sources can be treated in a physically acceptable way by the dispersion equation based on gradient transfer theory (K -theory)¹¹. Gravitational forces are usually neglected and only eddy turbulent transport is considered in the vertical direction. These assumptions are relevant for gases with approximately the same density as ambient air and for submicron particles. Larger particles must be treated separately.

If the dry deposition is discounted, simplified solutions of the diffusion equation lead to the Gaussian dispersion model, which is easy to handle and compare with experimental results, such as tracer studies. Deposition is easily accounted for by reducing the effective source strength as a function of distance. In this 'source depletion model'¹, the vertical concentration profile remains Gaussian and unchanged by the deposition at ground.

An alternative is to solve the dispersion equation with a flux boundary condition at the ground in agreement with gradient transfer theory¹²:

$$\{K(z) \partial c / \partial z\}_{z=z_g} = V_d(z_g) C(z_g)$$

where c is the concentration, z the height, $K(z)$ the eddy diffusivity as function of height, V_d the velocity of deposition and z_g the height of the reference surface. Solution of the dispersion

Table 1 Data extracted from studies of dispersion from a continuous point source 25 m above ground⁴

Distance Parameter	$H(m)$	1 km $K(m^2 s^{-1})$	R	$H(m)$	10 km $K(m^2 s^{-1})$	R	$H(m)$	20 km $K(m^2 s^{-1})$	R
Unstable	200	30.00	15.0	500	50.00	10.0	600	60.00	10.0
Neutral	70	2.50	4.0	190	4.00	2.0	210	4.50	2.0
Stable	25	0.05	0.2	60	0.10	0.2	75	0.15	0.2

H is effective plume height, K is the average diffusivity in the layer, and R is the discrimination ratio.

equation with this requirement results in a 'surface depletion model'^{8,9}, where the vertical concentration profile is modified by the loss of material at the ground. The decreased concentration at the ground in the surface depletion model, verified by experiments^{2,3}, results generally in decreased deposition, compared with the source depletion model.

A recent Gaussian surface depletion model⁵ shows that the source depletion model consistently overpredicts the surface air concentration and the deposition at downwind locations close to the source, and is consequently biased in the opposite direction for locations far from the source. The largest differences appear for low sources in stable stratification over surfaces with relatively large deposition velocities. Some parameters were found to be in error by factors of 3–4 at downwind distances of 10 km (ref. 5).

One-dimensional surface depletion models have been used for long distance travel^{3,6,7}. Considering the amount of airborne material after one day's travel during neutral stratification and with a deposition velocity of 3 cm s^{-1} , the source depletion model underestimates the total amount of airborne material by a factor of 10 compared with this surface depletion model⁶.

Studies with a two-dimensional pseudo spectral surface depletion model⁴ using physically realistic diffusivity and wind profile showed that the latter is important close to the source. Figure 1 shows the suspension ratio, that is the ratio between the vertical integrated airborne mass computed with and without deposition as a function of distance from a continuous point source at a height of 25 m. In the unstable (*a*) and neutral (*b*) case, the source and surface depletion models do not deviate significantly. The stable (*c*) case shows that the surface depletion model yields a lower suspension ratio than the source depletion model at distances $< 6 \text{ km}$ while the opposite is the case at larger distances. Further details on these studies will be published elsewhere⁴.

These results indicate that the deposition close to the source is underestimated and, in general, overestimated at medium distances with a source depletion model. But if a plume is dispersed in stable stratification and, at a distance of 20–30 km from the source, disperses to the ground due to, for example, mechanical turbulence and buoyancy induced by an urban area, the deposition is underestimated by the source depletion model by a factor close to 2. These results might be important for assessment of safety around industrial, such as nuclear, installations.

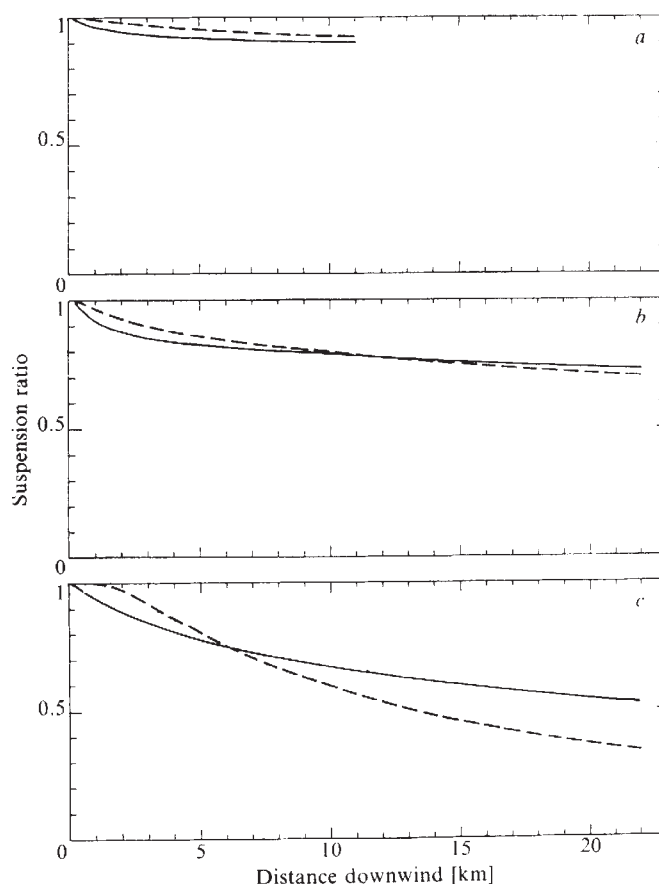
The deposition velocity used here: $V_d = 1 \text{ cm s}^{-1}$ is an average value for SO_2 . Submicron particles, such as sulphate-containing particles, deposit with an approximate velocity of one order of magnitude lower^{13,14}. The diffusivity profiles are relevant for rural country, while the diffusivity, for example, over cold water surfaces, is significantly decreased because of small aerodynamic roughness and cooling at the surface¹⁵. In general, both diffusivity and deposition velocity are functions of surface characteristics and meteorological conditions¹⁴.

The error introduced by the source depletion method is determined by the ratio between the eddy turbulent resistance and the resistance represented by the deposition velocity. A depth L can be determined by the ratio between an effective eddy diffusivity K , and the deposition velocity: $L = K/V_d$, where K is the average diffusivity over the depth H of the atmosphere containing the dispersed material. At large distances from the source, H is equal to the mixing height. At

small distances, $H \approx h + \sigma_z$, where h is the effective source height and σ_z is the standard deviation of the concentration distribution in the vertical direction.

If $L \approx H$, the resistance of the eddy transport is comparable to the deposition resistance, and a source depletion model will not introduce significant errors. This is shown to be the case in neutral and unstable atmospheric conditions with $V_d = 1 \text{ cm s}^{-1}$ (see Fig. 1 and Table 1). During stable atmospheric conditions, L becomes much smaller than H , and a surface depletion model should be used in order to avoid significant errors. A discrimination ratio $R = L/H$ is evaluated on the basis of present studies⁴ (Fig. 1 and Table 1). R can be interpreted as the ratio between a characteristic time for depletion of the depth H of the atmosphere by deposition $\sim H/V_d$, and a characteristic time for diffusion over the same distance $\sim H^2/K$; R is a function of source height and distance. Thus R should not be confounded with the turbulent Sherwood number of vertical surface layer mass transfer. Our studies show that only if $R > 1$, can the source depletion method be applied without significant

Fig. 1 Computed suspension ratio as function of distance from a source at a height of 25 m above ground. Solid line gives the values from a surface depletion model. Broken line gives the values from a source depletion model. Large discrepancies are seen only under the stable atmospheric conditions. *a*, Unstable; *b*, neutral; *c*, stable.



errors, while the surface depletion method can be applied for all values of R . This relation has not been previously taken properly into account^{1,10}.

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Chemical pretreatment and radial flow of ^{14}C in tree rings

THE prospect of very large increases in atmospheric CO_2 concentrations by fossil fuel combustion during the next century has prompted an active interest in CO_2 levels of the last century. One way of measuring these concentrations is by doing precise ^{14}C determinations in wooden tissue of known age. The first question to be answered, then, is whether wood grown during a certain year truly reflects atmospheric ^{14}C levels of that year. And if whole wood does not, what chemical treatment has to be given to it in order to isolate those substances that do reflect atmospheric ^{14}C at the time of formation of the ring. Attempts to answer this question have been made but the only conclusion that investigators^{1–5} generally agree on is that all resin fractions are to be distrusted and that the amount of contamination by material from other years of growth depends on tree species. But in some cases resin extraction by organic solvents does not seem to be sufficient^{1,2}. Having installed a new high precision ^{14}C counting system, to be described elsewhere, we looked at this question again, in view of our further tree ring measurements and the chemical treatment to be applied to our samples. We found that in the tree studied, an oak, radial movement of ^{14}C across ring boundaries is minor, even where the heartwood–sapwood transition takes place. If year to year

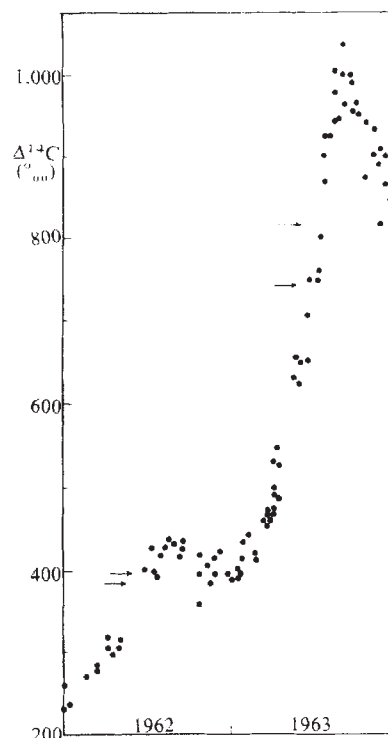


Fig. 1 $\Delta^{14}\text{C}$ in atmospheric CO_2 of the northern hemisphere. This figure is a compilation of atmospheric ^{14}C measurements carried out by Nydal, Vogel and Olsson during 1962–1963. $\Delta^{14}\text{C}$ of wood cellulose (arrows) is compared with values from Farmer and Baxter¹, the lower arrows in both years referring to Farmer and Baxter.

variations are small, the effect is negligible. Furthermore, cellulose and wood, treated successively with hydrochloric acid, sodium hydroxide and again hydrochloric acid, both seem acceptable for monitoring past ^{14}C levels, although they may not exactly refer to the same time of year.

Extensive hydrogen bomb testing raised the ^{14}C level of the northern hemisphere some 40% from 1962 to 1963. If any 'radial flow' of ^{14}C between successive tree rings exists, the effect is expected to emerge clearly in these year rings. We, therefore, selected wood from these years from the stem of an American oak (*Quercus rubra*), grown in the rural area of the province of Drente, the Netherlands (52° 45' N, 6° 50' E) that was felled by a severe storm in November 1972. In this tree the heartwood–sapwood transition took place in the rings of 1961–64.

Four different modes of chemical pretreatment were applied: (1) no pretreatment, that is combustion to CO_2 of whole wood; (2) treatment with 4% HCl at 80 °C during 24 h; (3) treatment with successive 4% HCl (80 °C, 24 h), 4% NaOH (80 °C, 24 h), and 4% HCl (80 °C, several hours), each step separated by thorough washing with demineralised water. This treatment is

Table 1 ^{13}C and ^{14}C determination of *Quercus rubra* after chemical pretreatment

Sample name	Laboratory identification	Pretreatment	Sample size (g)		$\delta^{13}\text{C}_{\text{PDB}}$ (‰)	$\Delta^{14}\text{C}$ (‰)
			before pretreatment	after		
AS 1963	GrN-8060	Cellulose	233	96	-23.50	820.6 ± 1.5
AS 1963	GrN-8061	AAA	116	46	-25.24	829.7 ± 1.2
AS 1963	GrN-8101	Acid	130	84	-24.45	809.3 ± 1.7
AS 1963	GrN-8102	Whole wood	72	72	-24.53	805.5 ± 3.5
AS 1962	GrN-8062	Cellulose	278	99	-24.45	389.2 ± 1.5
AS 1962	GrN-8063	AAA	150	59	-25.02	401.3 ± 1.6
AS 1962	GrN-8100	Acid	148	81	-25.41	412.6 ± 1.6
AS 1962	GrN-8099	Whole wood	91	91	-26.03	409.4 ± 1.6

routinely applied to most organic samples in our laboratory (AAA treatment); (4) preparation of α -cellulose, successively obtained by extraction with a 2:1 mixture of benzene-ethanol during several hours, rinsing with ethanol, bleaching by a NaClO_2 solution and acetic acid⁸, followed by treatment with 4% NaOH (80 °C, 24 h) and 4% HCl (80 °C, several hours). The last three steps again are separated by washing with demineralised water.

Before using methods (2), (3) or (4) the wood has to be chipped to matchstick size. The size of the final samples as they are measured is 50–55 l of CO_2 gas at NTP. The samples were kept under high pressure in small stainless steel containers. Therefore, special care had to be taken that the tiny amount of gas needed for ^{13}C determination had not undergone isotope fractionation with respect to the bulk of the sample. The results are given in Table 1 and plotted in Fig. 2, together with the results of Farmer and Baxter¹. The $\Delta^{14}\text{C}$ values are obtained by

$$\Delta^{14}\text{C} = ((A_s - 0.95 A_{ox}) / 0.95 A_{ox}) \times 1,000$$

where A_s and A_{ox} are the age and fractionation corrected ^{14}C activities of the sample and oxalic acid (samples to $\delta^{13}\text{C} = -25.0\text{‰}$, oxalic acid to -19.0‰).

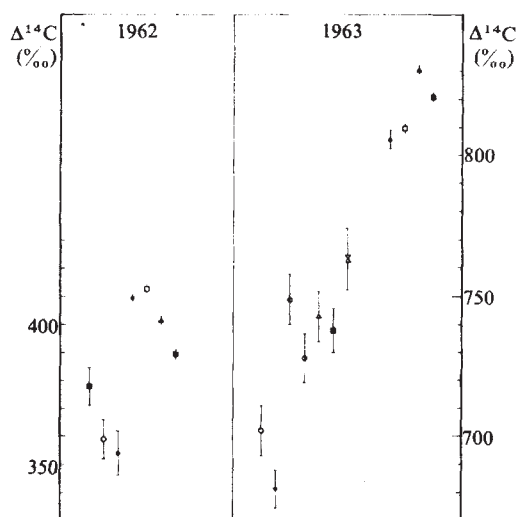


Fig. 2 $\Delta^{14}\text{C}$ in tree rings after different chemical pretreatments. The small error bars refer to this paper the larger to Farmer and Baxter¹: ● Whole wood; ■, cellulose; □, acid; ▲, AAA; ○, acetone; ●, acetone-petrolether; ×, lignin; △, acid-alkali; ○, acetone-acid-alkali.

The $\Delta^{14}\text{C}$ of whole wood from 1962 is 20‰ higher, that from 1963 15‰ lower than the respective cellulose fractions. With regard to a difference between the atmospheric ^{14}C levels in these years of about 400‰ this points to a 4–5% 'mixing' of organic material, presumably resins, across the 1962–63 boundary. Compared to the AAA pretreatment these figures are +10‰ and –25‰ respectively. Treatment with acid does not change this picture much. The AAA sequence, however, gives a result which is about 10‰ higher than cellulose both in 1962 and 1963 and when another, arbitrary ring was tried:

$$\begin{aligned} 1971 \text{ cellulose } \Delta^{14}\text{C} &= +496.4 \pm 2.8\text{‰} \quad \Delta^{13}\text{C} = -23.88\text{‰} \\ 1971 \text{ AAA } \Delta^{14}\text{C} &= +512.5 \pm 3.6\text{‰} \quad \Delta^{13}\text{C} = -25.27\text{‰} \end{aligned}$$

An explanation for this could be that the AAA treatment attacks earlywood more than it does latewood. The stratospheric leak occurring each year at our latitudes causes ^{14}C levels to be much higher in August than in early spring, at least several per cent during the 1960s. An alternative explanation is that the AAA treatment does not remove lignin or its fragments. Lignin is added to the cell structure in later stages of cell growth⁷. Full lignification of cells in *Pinus ponderosa*, for instance, occurs about a month later than cellulose formation. This hypo-

thesis is supported by the $\delta^{13}\text{C}$ values found, as lignin generally is more depleted in ^{13}C than cellulose. Also Wilson and Grinstead⁸, in searching for climatic information from stable isotopes in very thick tree rings, have found a displacement in time of the lignin curve against the cellulose curve.

Furthermore, Fig. 2 shows that Farmer and Baxter's $\Delta^{14}\text{C}$ values are consistently lower than ours. Cellulose for instance is $12 \pm 7\text{‰}$ lower in 1962 and $83 \pm 8\text{‰}$ lower in 1963. This cannot result from a latitudinal effect, as Nydal has shown it to be virtually non-existent⁹. Moreover, their tree has grown at almost the same latitude (Glengarry, Scotland, 57° N) as ours. Both trees grew in rural environments. In our view, the observed effect follows either from a shift in time of major growth, that is, the Glengarry tree grew earlier during the year than the Drente tree, or local recycling of CO_2 is involved, that is, the incorporation in the ring of CO_2 from decaying organic matter in the forest. The rate of increase of atmospheric ^{14}C levels was 40‰ per month and 90‰ per month until August in 1962 and 1963 respectively (Fig. 1). This observation supports the first hypothesis but does not exclude the second. The second hypothesis is supported by the fact that the Drente tree was planted in 1880 and did not grow in a forest, while the Glengarry tree was only planted in 1937 in a forest.

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Unusual room temperature afterglow in some crystalline organic compounds

STRONG room temperature phosphorescence studies of certain doped solid systems^{1–4} have shown that the characteristic phosphorescence peak wavelengths agree with those established for the corresponding dopants dissolved in 77K systems; the room temperature lifetimes being smaller by a factor of 1.5–2, depending upon the nature of the matrix. We have recently observed an unusual new phenomenon—a weak afterglow from certain of the dopant compounds including carbazole, dibenzothiophene, dibenzofuran and triphenylene when examined as pure crystalline solids. This weak afterglow has different peak wavelengths and lifetimes, but in dilute solid solution form in room temperature resin systems there is no trace of the weak afterglow properties. Melting and resolidification of these pure aromatic compounds do not affect the weak afterglow, and, after vacuum sublimation of carbazole, the afterglow is still observed from the purified product. We believe that we can rule out an impurity effect and this view is reinforced as the afterglow intensifies after recrystallisation from ethanol. As the crystals probably improve on recrystallising, the crystal quality may play a role in this phenomenon.

The phosphorescence spectrum of a dilute (1%) solid solution of carbazole in a thermoset resin at room temperature is shown in Fig. 1b (recorded using an EMI

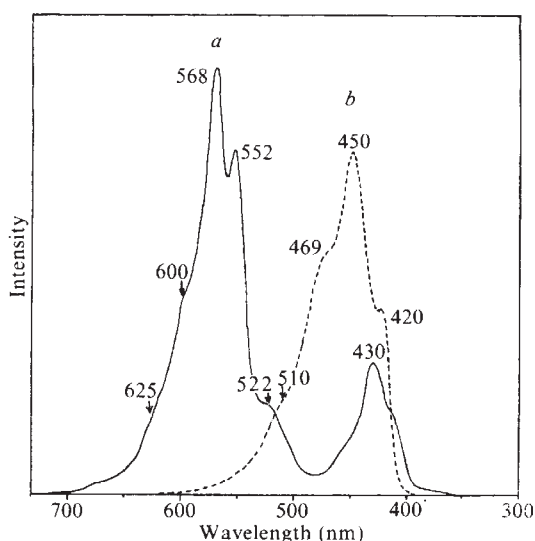


Fig. 1 *a*, Room temperature afterglow spectrum of crystalline carbazole. *b*, Phosphorescence spectrum of 10^{-2} Molal solid solution of carbazole in a thermoset resin at a gain factor of 0.05 relative to *a* at the same setting of the spectrophosphorimeter.

photomultiplier type 6256). The main peaks at 420, 450 and 469 nm correspond well with the phosphorescence emission peaks in *n*-heptane at 77K (ref. 5). The room temperature lifetimes of the 420 and 450 nm peaks are 3.8 and 4.85 s respectively, which is a reduction by a factor of 2 compared with the 77K system. The weak afterglow spectrum is also shown in Fig. 1*a*, at a gain factor of 20 for 100% solid crystalline carbazole at an identical setting of the spectrophosphorimeter. This spectrum shows peaks at approximately 430 nm and also at 522, 552 and 568 nm wavelengths. The 430 nm peak has a decay time which is faster than the chart recorder response, 0.3 s. The other peaks at 552 and 568 nm show mean lifetimes of 0.7 and 0.88 s respectively. Figure 2*b* shows log intensity plotted against time for the strong 450 nm room temperature phosphorescence of the carbazole solid solution in resin and Fig. 2*a* shows the corresponding curve for the weak afterglow peak at 552 nm. As the plot for the weak afterglow is a straight line, it is possible that the weak afterglow, having a first-order decay, has origins in a metastable state, as does the normal 450 nm triplet.

Fig. 2 Plots of $\ln I$ against time. Plot *a* for 552 nm peak of afterglow (see Fig. 1*a*); *b* for 450 nm phosphorescence peak (see Fig. 1*b*).

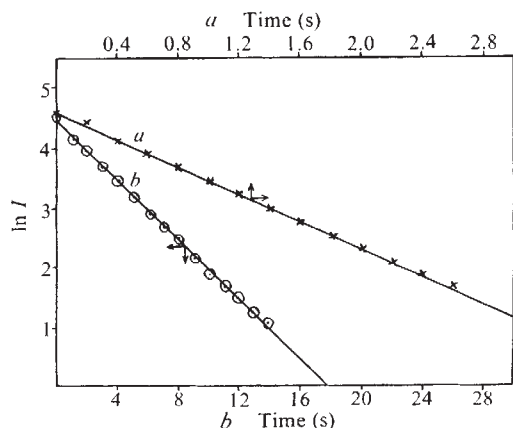


Figure 3*a* shows the total emission spectrum of crystalline carbazole, which is mainly fluorescence, with a negligible phosphorescence above 500 nm. The totally fluorescent emission spectrum of a 10^{-2} Molal solid solution of carbazole in PMMA is shown in Fig. 3*b* (both curves were recorded as observed with an EMI photomultiplier type 9524B). Figure 3*a* for crystalline carbazole, was recorded at half the slit width used for Fig. 3*b*, giving the latter curve a gain factor of times 2. The excitation for both spectra was achieved by the 312 nm mercury lamp line through a grating monochromator. Comparing the curves, Fig. 3*b* for the solid solution shows emission typical of molecular carbazole in dilute solutions in solvents such as ethanol and cyclohexane, apart from some self-absorption at the shoulder at 343 nm (ref. 6). Figure 3*a* for crystalline carbazole, however, has a much broader peak which is red-shifted by 67 nm compared to the higher peak of curve *b* and, in addition, curve *a* does not show any well defined vibrational structure in contrast to the other three emission curves shown in Fig. 1 and Fig. 3*b*. We note that although the fluorescence peak intensity at 423 nm in Fig. 3*a* is about 2 or 3 orders stronger as compared with the afterglow peak intensity at 430 nm in Fig. 1*a*, the peak shapes and position are very similar in both cases. This result indicates that the 430 nm peak in the weak afterglow spectrum has origins from emissive states electronically similar to those yielding fluorescence in crystalline carbazole.

The characteristic strong room temperature phosphorescence properties of carbazole in the thermoset resin (Fig. 1*b*) shows that this is radiative emission from the triplet state. But from the magnitude of the peak intensity of the room temperature phosphorescence, we suspect that the triplet state may be populated by a donor species. The crystalline form of carbazole, however, shows no blue phosphorescence, indicating that either insufficient numbers of normal triplet states are populated, or that the energy is channelled to lower emissive states responsible for the peaks at 522, 552 and 568 nm (see Fig. 1*a*).

Reappraising the afterglow (curve *a*) and phosphorescence (curve *b*) spectra in Fig. 1, we note that the vibrational spacings of the peaks are similar in both cases. The overall shapes also match each other, apart from a magnitude inversion of the 552 and 568 nm peaks compared to the 450 and 469 nm peaks.

Wavelengths of various peaks and shoulders are listed in Table 1*a*. There is good correlation between the two columns, all peaks in column 1 being red-shifted by approximately 90–100 nm. The peaks at 522, 552 and 568 nm show first-order decay rates of afterglow intensity after the illumination is stopped; however, the lifetime is of an order of magnitude smaller compared to the 450 nm phosphorescence peak (≈ 0.7 s, as opposed to ≈ 4.5 s). These results indicate that the afterglow is due to an emission to S_0 of the monomer or to a vibronically similar ground state. Furthermore, T_1 (metastable state giving weak afterglow) lies below T_1 , the monomer triplet state (the difference being $\approx 4,106 \text{ cm}^{-1}$). Comparisons of the various peaks and shoulders of fluorescence emission spectra of crystalline carbazole and a 10^{-2} Molal solid solution of the same compound in PMMA are shown in Table 1*b*. Apart from a correlation of the two weak shoulders on the long wavelength tail of the emission, there are great discrepancies in the two spectra. This indicates that the excited state in crystalline carbazole (probably a first excited singlet, S'_1) has facilities for vibronic energy dissipation and, furthermore, the strong emission at 423 nm is unique to the crystalline samples. An explanation for these results could be the presence of dimers in the crystalline carbazole, or the formation of an excimer during photoexcitation. Excimer emission from liquid solution is well known for pyrene⁷

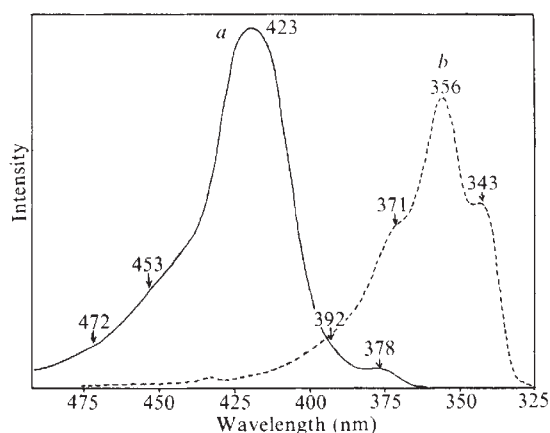


Fig. 3 Fluorescent emission spectra of carbazole. *a*, Crystalline carbazole, *b*, 10^{-2} Molal solution in a thermoset resin at a gain factor of 2 compared to *a*. Excitation for both spectra provided by 312 nm line through a grating monochromator from a high-pressure mercury lamp.

which shows a broader and red-shifted spectrum compared to the pyrene monomer emission. We think that random variations of orientation and spatial variations which may be responsible for such broadening in the liquid state would be inhibited in solid crystalline carbazole. The relatively sharp peaks compared to those for liquid state dimers⁷ in the fluorescence and weak afterglow spectra of crystalline carbazole are thus not altogether surprising.

Our results show, therefore, that the monomer in solid solution exhibits normal emission, corresponding to the first excited singlet state and the first excited triplet state. In the crystalline state of pure carbazole, the corresponding emissions of fluorescence and of weak afterglow are completely different, the fluorescent spectrum being broad and showing little or no vibrational structure, indicating energy dissipation from this excited state. The afterglow spectrum shows the unmistakable vibrational bands of transitions between two relatively stable states. We propose that the weak afterglow emission originates from the triplet state of a dimer or carbazole pair. The fluorescence of the crystalline carbazole would be expected to originate in the excited singlet state of the same species, however, the spectral broadening suggests some excimeric character, implying a less stable pairing in the excited singlet as compared with either a dimer triplet state or a dimer ground state.

Table 1 Comparison of afterglow and fluorescence emissions

<i>a</i> Afterglow or phosphorescence emission	
Crystalline afterglow possibly dimer or excimer $\lambda(T_1)$	Dilute glass phosphorescence monomer $\lambda(T_1)$
522	420
552	450
568	469
600	510
625	—
<i>b</i> Fluorescence emission	
Crystalline possibly dimer or excimer $\lambda(S_1)$	Dilute glass monomer $\lambda(S_1)$
379	—
392	—
423	—
—	343
453	356
473	371

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Utilisation of soapstone in Labrador by Indians, Eskimos and Norse

MUCH of our knowledge of prehistoric peoples of North America comes from the study of their utilisation of imperishable lithic (geological) resources. In some cases geochemical differences in the origin of a particular lithic material have resulted in trace element differences which can be used to characterise the geological source of this material¹. Both geochemical and archaeological studies indicate that the rare earth element (lanthanide) distributions in soapstone (steatite) can be used to characterise this commonly used lithic material^{2–4}. The rare earth elements (REE) in artefacts from Labrador and Newfoundland, measured by instrumental neutron activation analysis and reported here, suggest that the same soapstone sources have been used by Indians, Eskimos, and Norse at various times over the past 4,000 yr.

Apart from the importance of cultural history in general, Labrador, because of its location at the juncture of the arctic and subarctic zones, has an intriguing prehistory. The environmental and cultural boundaries in this region have been studied as they shifted through time^{5–7}. The Indians and Eskimos who occupied this area at various times utilised soapstone in characteristic ways. For instance, the Indians (Montagnais–Naskapi) who were present in southern Labrador 7,500 yr ago made soapstone plummets (fishing sinkers?) which have been found at Late Maritime Archaic (4,000–3,500 yr BP) sites in central and southern Labrador as well as Newfoundland. Dorset Eskimos (who first appeared in central Labrador from the north about 4,000 yr ago) used soapstone for oil lamps, cooking pots and several types of amulets. These artefacts are found in various Dorset sites from Newfoundland north which range in age from 2,500–1,000 yr BP. Later, in Labrador Eskimo times (500–150 yr BP) soapstone was extensively used for posts and lamps until replaced by European technology. A soapstone spindle whorl, found at L'anse aux Meadows, near the northern tip of Newfoundland, is part of the evidence for this early European settlement in North America^{8–9}. Similar artefacts are common in Norse sites in Iceland and northern Europe.

Soapstone, having discrete and localised sources, could not have been available to all of these groups in one locality. Consequently, trade or travel to secure artefacts or raw materials must have been common within and between cultural boundaries. The small size of most soapstone quarries and the lack of information about quarries during the past century in Labrador makes many of the source locations difficult to find. Nevertheless, matching artefacts from the same, but unknown, quarry does provide valuable information about resource procurement. As each geological deposit of soapstone may have formed under different conditions of contact or regional metamorphism from a variety of older rock types, trace element

concentrations vary considerably between different areas. Having analysed over 50 sources of soapstone in eastern North America, we have shown that each formation can be characterised by its trace element contents^{3,10}. In particular, the rare earth elements ($Z = 57-71$) vary in both absolute and relative abundances. The differences can be shown graphically by plotting the normalised concentration of the particular rare earth element (obtained by dividing its concentration in the soapstone by that found in chondritic meteorites) as a function of atomic number³. The curves (REE patterns) reflect the slightly different geochemical behaviour of these elements which result from differences in ionic radii and in the case of Eu, the ionic charge.

Each geological formation may have rocks which vary considerably in their mineralogies, but if they contain sufficient quantities of talcose minerals (such as talc, chlorite, antigorite, and tremolite) they have the physical characteristics which would have allowed them to be utilised. While we have observed that some of the trace elements (for example, Fe and Cr) do depend upon the mineralogy, it seems that the REE do not. Figure 1 shows three examples of the variations observed in REE patterns for soapstone from a particular geological region. Based upon the analysis of between 5 and 20 samples from these and several other individual geological formations, we conclude that the REE patterns are essentially the same in shape (are parallel) but differ in absolute magnitude. For REE concentrations which are about an order of magnitude greater than the chondritic concentrations, the magnitude of the differences observed between samples is about a factor of 2. When the REE concentrations in a soapstone formation are the same as or lower than the chondritic concentrations, the differences between samples from the same source are larger, differing by as much as a factor of 5, but the patterns are approximately parallel. This gives some insight into how well an artefact might be expected to match a sample from a quarry in order to establish the quarry as the source region.

Matching the REE patterns in soapstone artefacts to the source of the raw material is based on the assumption that the quarry can be located and sampled. Although over 50 different soapstone source regions (varying considerably in size) have been differentiated on the basis of their REE patterns, the analysis of artefacts from eastern North America suggest that others have not been found. This has been a particularly difficult problem because the analysis of over 60 Eskimo and Indian artefacts grouped together, according to their REE patterns, suggest that at least eight soapstone sources were used over the past 4,000 yr. But only four of these have been located. Two quarry areas have been sampled in Labrador, one at Freestone Harbour in the Davis Inlet area south of Nain and the other at Moores Island near Okak, north of Nain. On the island of Newfoundland the large outcrops at Fleur-de-Lys were sampled, as well as an outcrop a mile from the L'anse aux Meadows site (provided by the Conservation Division of Parks, Canada).

The L'anse aux Meadows quarry is of particular interest as it may have been the source of the Norse spindle whorl (Fig. 2). Other use of the L'anse aux Meadows quarry is indicated by a plummet, from the 4,000 yr old Maritime Archaic Indian site at Rattlers Bight in the Hamilton Inlet region, which also has a matching REE pattern¹¹. This relatively distant source was not the most common one for the Indians in this region as most of the plummets from this site have the same REE patterns, but the pattern does not match any of the quarries sampled. One might assume a more local source. In another Maritime Archaic site further north at Windy Tickle (Davis Inlet) the only two plummets have REE patterns which match a nearby soapstone outcrop at Freestone Harbour.

By 2,600 yr BP the Dorset Eskimo culture had advanced into central and southern Labrador and by 2,000 yr BP, into Newfoundland. Dorset people used some of the same quarries or quarry locations as the earlier Maritime Archaic Indians. The Freestone Harbour soapstone was used extensively in the Nain

and Okak areas between 2,700–1,400 yr BP. Soapstone from the Okak region (Moore's Island) was another relatively local source used extensively by the Dorset Eskimos in the Nain area based upon the matching of REE patterns in bowls to those of the quarry (Fig. 2). There was also the rare utilisation (10% of the samples) of the soapstone from the two quarries in Newfoundland. The problem still remains that nearly half of the 30 Dorset artefacts from central Labrador do not match any of these four quarries.

The method of transmission of information about soapstone quarries within the different Indian and Eskimo cultural

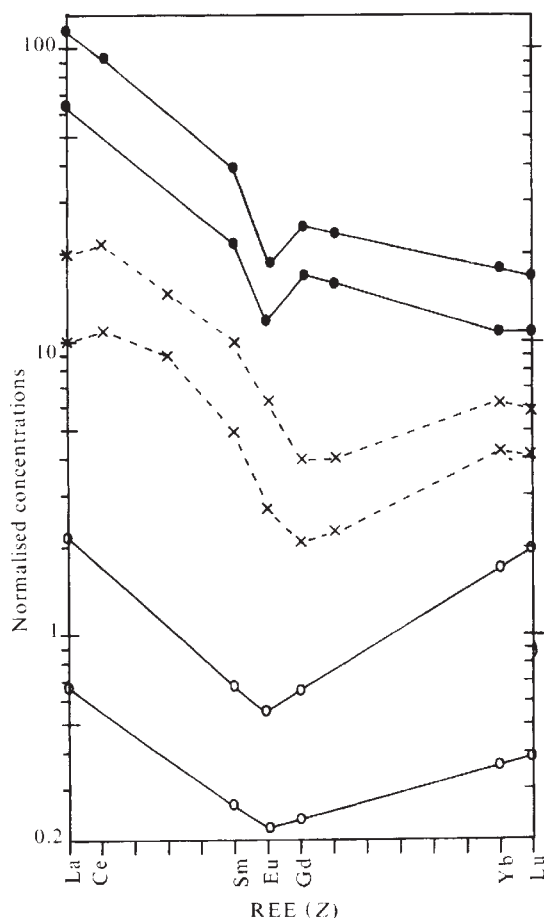


Fig. 1 Chondrite normalised rare earth element (REE) distribution patterns of some soapstone outcrops in Virginia and Crete. The Virginia samples are from the Albemarle–Nelson County region and the two lines (X) give the extremes of the range for 15 different samples analysed. The two lines (●) define the range of 14 samples from a relatively large region in Crete which is assumed to be a single large soapstone formation. The patterns (○) noted as Crete B define the range observed for 13 samples from the Goniae region of Crete. In all cases the other samples are approximately parallel to the curves shown but differ somewhat in magnitude within the range defined by these curves. This gives an indication of what type of variations might be expected for artefacts from a particular quarry.

sequences or between ethnic groups at times of culture area changes is not known. Oral tradition must have been important during periods of continuity, but prehistoric peoples (and Norse as well) must have been keen observers of geology. Prehistoric people of Labrador used many different lithic materials, most of whose source locations are unknown today. It is unfortunate that Western culture replaced native traditions so rapidly and completely before these observations could be recorded. We are now confronted with the difficult task of rediscovering the sources of many culturally important lithic materials for use in studying cultural movement and contact. The use of REE patterns to match artefacts to the geological source of the

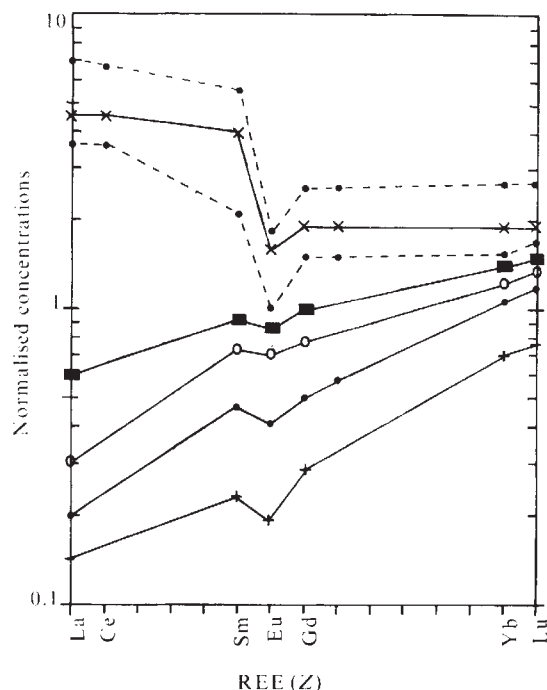


Fig. 2 Chondrite normalised REE patterns for two quarries and four artefacts interpreted as originating in these quarries. Quarry L (●) is a sample from a soapstone outcrop in northern Newfoundland near L'anse aux Meadows and the Norse artefact (■) is the spindle whorl from the L'anse aux Meadows site. The Indian artefact (○) is a plummet from a Maritime Archaic site at Rattler's Bight in Labrador while the Eskimo artefact (+) is from a rectangular cooking vessel from a Dorset Eskimo site in the Nain region further north. The dashed curves (●) outline the range of three samples of soapstone from outcrops in the Okak region (north of Nain) and the Eskimo artefact (×) is another cooking vessel from the same Dorset site in the Nain region.

soapstone provides an important new tool for the study of lithic resource procurement. Most of the soapstone tested from prehistoric sites to date seems to be from local quarries. The presence of occasional exotic materials argues for the existence of exchange networks maintained by social rather than economic need.

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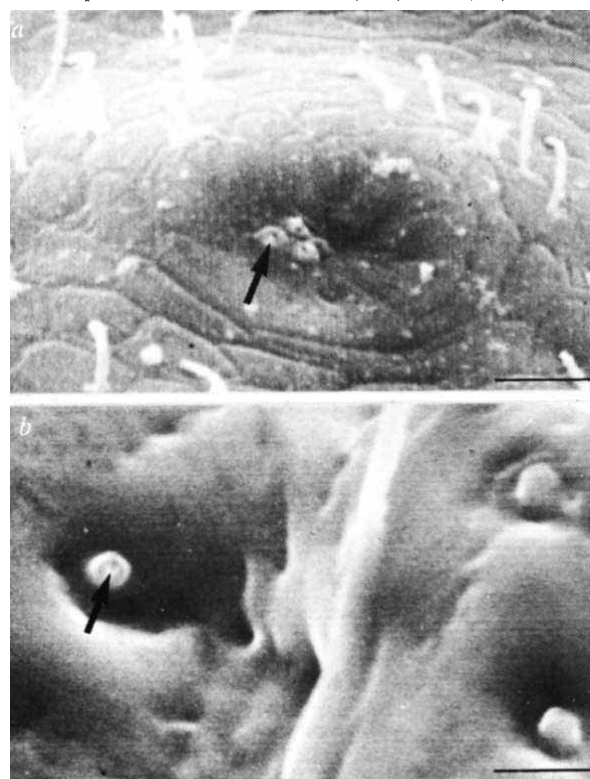
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Inhibition of oesophageal peristalsis in the lobster after chemical stimulation

PREVENTION of hyperphagia by some mechanism is essential to feeding animals. To date relatively few such control mechanisms have been studied in detail. Termination of feeding by internal inhibitory feedback from stretch receptors is documented for *Phormia regina*¹, *Locusta migratoria*², *Chortoicetes terminifera*³ and *Aplysia californica*⁴. Chemosensory adaptation has been suggested for *Phormia*¹ and *Chortoicetes*³, and in *Locusta* activation of foregut stretch receptors will, through a nervous and hormonal pathway, close the terminal pores of chemosensory sensilla on the maxillary palps⁵. We present here evidence showing that stimulation of a pair of oesophageal contact chemoreceptor organs can slow the ingestion rate and terminate feeding in *Homarus gammarus*.

The anterior oesophageal sensors (AOS) were first described in *Homarus* by Allen⁶ and were described in more detail in the crayfish *Astacus leptodactylus* by Orlov⁷ (for a review of the sensory innervation of the foregut of decapod crustaceans see ref. 8). These sensors are bilaterally symmetrical and their cuticular endings are situated in the clefts between the anterior and lateral lobes of the oesophageal/cardiac sac valve (data not shown). Each anterior oeso-

Fig. 1 Scanning electron micrographs of the cuticular modifications associated with the anterior oesophageal sensor. *a*, Grouped endings located in depressions in raised hillocks on the lateral wall of the anterior lobe of the oesophageal/cardiac sac valve. *b*, Single endings present deep in the cleft between the anterior and lateral lobes of the valve. It is possible that the sensilla have terminal pores (arrowed). The single endings bear a marked resemblance to those described for contact chemoreceptors in insects^{9,10}. Scale bar, 10 µm in *a*, 4 µm in *b*.



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phageal sensor is innervated by a dorsal branch of the ipsilateral superior oesophageal nerve (SON) and can be divided readily into two populations of receptor neurones; 1, 50–60 bundles of 3–5 small (15–20 μm long axis) uni-terminal bipolar neurones; and 2, 250–300 similar small neurones which are present singly. The cuticular modifications associated with the dendrites can be divided similarly (Fig. 1). In addition there are between two and five large (60–80 μm long axis) bipolar neurones whose axons also travel in the anterior oesophageal sensor nerve and whose long dendrites travel over the surface of the cardiac sac and oesophagus in the region of the valve.

Recording and stimulation of selected nerves was carried out with silver wire hook electrodes using conventional techniques. In all experiments the bathing medium was clean, cool seawater. The organ was chemically stimulated with an extract of *Mytilus edulis*. The gills and mantle of a fresh mussel were homogenised in approximately the same volume of seawater. This was applied directly on to the anterior oesophageal sensor with a glass pipette inserted through a hole cut in the cardiac sac. The afferent axons of other chemoreceptors in the area were cut.

The oesophagus undergoes peristalsis for some time after initial dissection, and recordings from the superior oesophageal nerve during this time reveal a complex rhythmical burst which can be shown to occur during oesophageal dilatation. This burst was used as an indicator of oesophageal dilatation during peristalsis. The frequency of bursting is relatively constant at any one time but varies between 0.1 Hz and 0.33 Hz. Suprathreshold electrical stimulation of the anterior oesophageal sensor nerve with a train of rectangular pulses of width 0.6 ms and with a pulse interval of 500 ms has two effects (Fig. 2). In this preparation the burst frequency dropped from its original level (about 0.28 Hz) to approximately 0.1 Hz; this was accompanied by a drop in the number of spikes in each burst. On cessation of stimulation the burst frequency and the number of spikes per burst increased but did not reach their original level. Application of *Mytilus* extract directly on to the anterior oesophageal sensor mimics the effect of electrical stimulation of the sensory axons by reducing the burst frequency (Fig. 3), although there is no reduction in the number of spikes per burst. Continued application can terminate bursting, and to be effective the extract must be closely and continuously applied.

The anterior oesophageal sensors are classified as contact chemoreceptors on morphological grounds and direct ex-

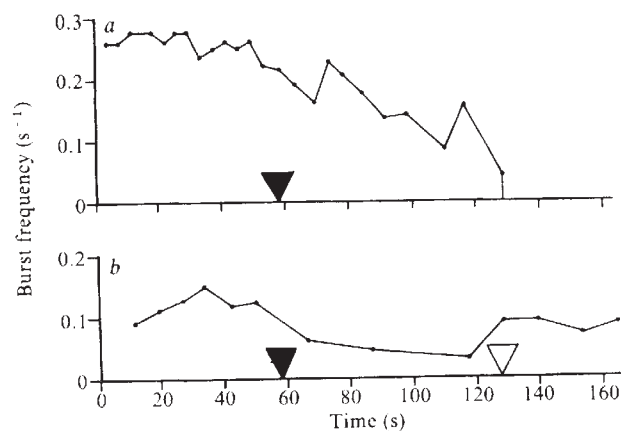


Fig. 3 Effect of chemical stimulation of the anterior oesophageal sensor on rhythmic bursting in the superior oesophageal nerve. (a) and (b) are different experiments. Application of *Mytilus* extract (solid arrow) slows and stops bursting (a). In (b) burst frequency increases on removal of the pipette (open arrow) but without washing the organ.

perimental evidence is not yet available. The positions of the receptor organs are such that they become available for stimulation only when the cardiac sac is filled to capacity and the oesophageal/cardiac sac valve is stretched open. The solitary endings lie deep in the cleft between the lobes of the valve while the grouped endings spread on to the lateral walls of the anterior lobe. It is unlikely that these positional differences reflect functional differences, the more complex arrangement of the grouped endings probably serving to prevent causal stimulation during feeding. The observed difference between electrical and chemical stimulation can be explained by the presence in the anterior oesophageal sensor nerve of the axons of the small group of large bipolar neurones. These are presumptive mechanoreceptors that may respond to stretch in the region of the oesophageal/cardiac sac valve and could also be involved in the control of the peristaltic rhythm.

These observations suggest an alternative control mechanism for feeding processes, dependent upon chemical rather than mechanical feedback to higher centres. It is possible that such methods of controlling food ingestion will be found more commonly, especially amongst decapod crustaceans.

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A *Rhizobium* mutant incapable of nodulation and normal polysaccharide secretion

THE surface carbohydrates of *Rhizobium*, including both the lipopolysaccharide and the extracellular polysaccharide, are thought to participate in the infection and nodulation of legumes by these Gram-negative bacteria^{1–3}. We report here the isolation of a mutant of *Rhizobium leguminosarum* which produces diminished amounts of extracellular polysaccharide

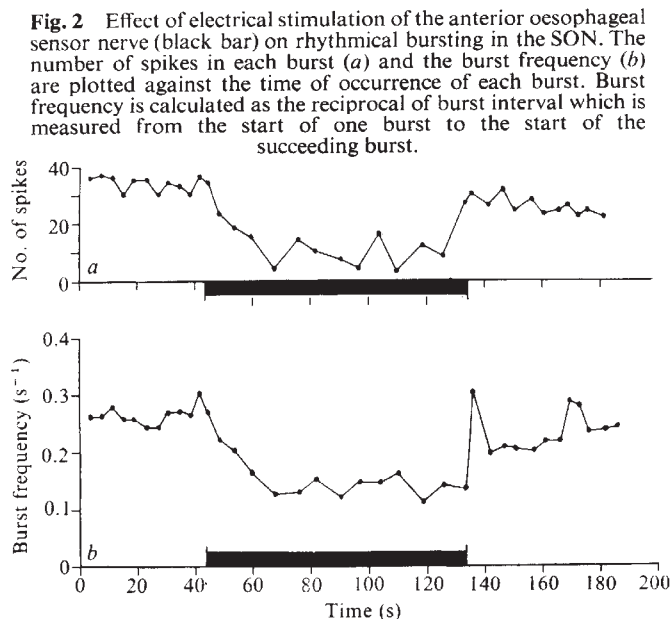


Fig. 2 Effect of electrical stimulation of the anterior oesophageal sensor nerve (black bar) on rhythmic bursting in the SON. The number of spikes in each burst (a) and the burst frequency (b) are plotted against the time of occurrence of each burst. Burst frequency is calculated as the reciprocal of burst interval which is measured from the start of one burst to the start of the succeeding burst.

(that is, the carbohydrate material present in cell-free filtrates of bacterial cultures) and which fails to nodulate pea, its normal host.

Two antibiotic resistance markers were introduced, by spontaneous mutation, into *R. leguminosarum* strain 128c53 (obtained from J. C. Burton of Nitragin Co.). Approximately 10^{10} cells of this strain growing in minimal liquid medium⁴ were concentrated by centrifugation. The cell pellet was resuspended in 0.1 ml of medium and spread on a minimal agar plate⁴ containing 100 µg of streptomycin sulphate per ml. A single colony arising on this medium was purified twice by streaking minimal medium plus streptomycin. This streptomycin resistant (*str*) derivative of 128c53 was then grown in liquid medium, concentrated, and spread on solid, defined medium containing 100 µg streptomycin and 20 µg rifampicin per ml. A purified single colony resistant to both streptomycin and rifampicin (*rif*) was checked for its ability to nodulate pea seedlings. This *str* and *rif* derivative of 128c53 was then used to isolate five strains of bacteriophage (differing in plaque morphology) from a mixture of mid-western soils according to the procedure of Vincent⁵.

Spontaneously arising mutants of *R. leguminosarum* 128c53 *str* and *rif* which produce decreased amounts of extracellular polysaccharide were obtained by passing late stationary phase cultures of this strain through 1.2-µm Millipore filters, adding an equal volume of fresh medium, and allowing the cells to grow into late stationary phase again. This process was repeated four times over approximately 30 d. These bacteria were then diluted and spread on solid media to observe colony morphology. Over 90% of the colonies arising on such plates were small and seemed to lack the heavy slime produced by the starting strain, 128c53 *str rif*. Seven of these small colonies were purified and examined further.

Table 1 Extracellular polysaccharide production by *R. leguminosarum* 128c53 *str rif*, by EXO-1, and by a revertant of EXO-1, EXO+4

Strain	Carbohydrate produced (glucose equivalents ($\text{g} \times 10^{-12}$) per viable count)
128c53 <i>str rif</i>	32.0
EXO-1	1.14
EXO+4	31.0

Cells were shaken for 6 d in liquid medium⁴ at 24 °C until a density of approximately 10^8 ml⁻¹ was obtained. Viable counts were then determined by plating appropriate dilutions on solid agar medium⁴. Extracellular material was prepared as follows. Cultures were centrifuged for 30 min at 10,000 r.p.m. The supernatant solutions were passed through 5-µm Millipore filters and then concentrated under reduced pressure and at 40 °C to give one-tenth of the original volumes. The concentrated supernatant solutions were dialysed and lyophilised. The resulting powders were dissolved in 0.2 M imidazole buffer, 0.1 M NaCl, pH 7, and assayed for carbohydrate content by the anthrone method⁶.

Bacteria from all seven small colonies were resistant to streptomycin and rifampicin and sensitive to bacteriophage 1-5 (see above). The strain producing the smallest colonies, designated EXO-1, was chosen for further study.

EXO-1 has a doubling time, both for viable counts and optical density, identical to its parent *R. leguminosarum* 128c53 *str rif* (data not shown). This indicates that EXO-1 does not produce small colonies due to a defect which slows its growth. Extracellular carbohydrate was determined as described in Table 1. On a per cell basis, EXO-1 culture filtrates contain approximately 5% of the amount of anthrone-positive material present in culture filtrates of 128c53 *str rif*. EXO-1 and four similar mutants derived from 128c53 *str rif* by the procedure described above were used to inoculate pea seedlings (Table 2). All five of these strains failed to nodulate Alaska pea.

Indirect evidence suggests that the lipopolysaccharide component of the cell wall of *Rhizobium* may be involved in

Table 2 Nodulation tests

Strain	No. of nodules per plant
<i>R. leguminosarum</i>	280
<i>R. leguminosarum</i> 128c53 <i>str rif</i>	270
None	0
Extracellular polysaccharide	
Deficient derivatives of	
<i>R. leguminosarum</i> 128c53 <i>str rif</i>	
EXO-1	0
EXO-2	0
EXO-3	0
EXO-4	0
EXO-5	0
Extracellular polysaccharide	
revertants of EXO-1	
EXO+1	260
EXO+2	220
EXO+3	230
EXO+4	220
EXO+5	240

All values are the average of at least three separate experiments, each of which contained at least three plants. Peas were surface sterilised by treatment with 70% ethanol for 5 min followed by treatment with 30% hydrogen peroxide for 30 min. These surface-sterilised peas were soaked overnight in sterile Fahraeus solution⁷ and then planted in sterile vermiculite. The plants were grown using a 14-h light/10-h dark cycle at 22 °C day temperature and 18 °C night temperature. Illumination was 2,500 foot candles. Bacteria were grown to a density of approximately 10^8 ml⁻¹ (ref. 4) by shaking at 24 °C. Cultures were then centrifuged and resuspended in an equal volume of distilled water. Ten-day-old Alaska pea seedlings were inoculated with 10 ml of these bacterial cultures. The roots of these plants were inspected 18 d later. Extracellular polysaccharide producing revertants of EXO-1 were obtained by spreading approximately 10^6 cells of this strain on minimal agar plates⁴. Slime-producing colonies, which arose on these plates at a frequency of approximately 10^{-5} , were isolated and purified.

host-symbiont selection². We therefore examined the lipopolysaccharide of EXO-1 to determine whether this cell surface component is altered by the mutation which reduces extracellular polysaccharide production in this strain. By three criteria, the lipopolysaccharide of EXO-1 seems very similar, if not identical, to that of its parent, 128c53 *str rif*. First, as mentioned above, EXO-1 and 128c53 *str rif* are sensitive to the same bacteriophage. Since most receptors for bacteriophage are located in the lipopolysaccharide⁸, the similarity of phage

Table 3 Glycosyl composition* of the lipopolysaccharides of *R. leguminosarum* 128c53 and 128c53 *str rif* EXO-1

Sugars	<i>R. leguminosarum</i> strains	
	128c53 %	128c53 <i>str rif</i> EXO-1 %
Rhamnose	24	21
Fucose	33	27
Mannose	18	23
Galactose	4	3
Glucose	1	1
2-amino-2, 6-dideoxyhexose	2	3
Uronic acid	14	14
KDO	10	8

Aldoses and amino sugars were analysed as their alditol acetates. The lipopolysaccharides were hydrolysed for 60 min at 121 °C in 2 M trifluoroacetic acid. The resulting monosaccharides were converted to their corresponding alditol acetates and analysed by combined gas chromatography-mass spectrometry¹². 2-Keto-3-deoxycaranoic acid (KDO) was determined using the thiobarbituric acid colorimetric assay¹³ with the ammonium salt of KDO (Sigma) as the standard. Uronic acid was determined using the *m*-hydroxybiphenyl assay¹⁴ with galacturonic acid as the standard.

*The sugar composition is given as per cent of total sugar. In strain 128c53, the total sugars account for 63% of the lipopolysaccharide mass. In 128c53 *str rif* EXO-1, the sugars account for 27% of the lipopolysaccharide mass.

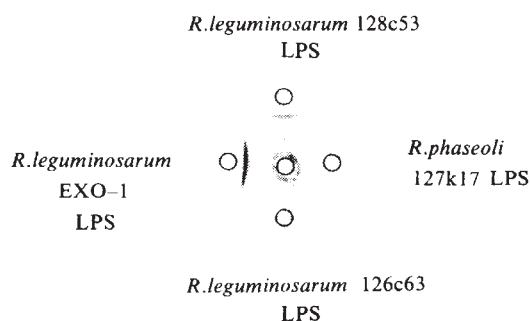


Fig. 1 Immunochemical analysis of the lipopolysaccharides from various *Rhizobium* strains. Antisera (centre well) prepared against the lipopolysaccharide purified from *R. leguminosarum* 128c53 forms a precipitin band when diffused against the lipopolysaccharide from this strain and when diffused against the lipopolysaccharide from its extracellular polysaccharide deficient derivative, EXO-1. The antisera does not cross-react with lipopolysaccharide purified from another strain of *R. leguminosarum* or with lipopolysaccharide purified from a strain of *R. phaseoli*. Each well contains 15 μ l of a 2 mg ml⁻¹ solution of the indicated lipopolysaccharide. In general, the lipopolysaccharides prepared from *Rhizobium* are immunochemically strain and species specific (ref. 9 and R.W.C., C. Napoli, R.E.S. and P.A., in preparation). Lipopolysaccharides were prepared as previously described² with the following modification. The aqueous phase of a phenol-water extract of *R. leguminosarum* 128c53 is viscous and must be ultracentrifuged at 100,000g for 3 h before use of the Agarose A 1.5m column. The other lipopolysaccharide preparations were not as viscous and were purified without the ultracentrifugation step. Before the ultracentrifugation or passage through sizing columns, the lipopolysaccharide preparations were incubated overnight (17 h) at room temperature with 1 mg each of DNase I (Sigma) and RNase A (Sigma) to depolymerise the nucleic acids. The final lipopolysaccharide preparations do not have a λ_{max} between 230 nm and 320 nm and form typical opalescent solutions when dissolved in water at a concentration of 1 mg ml⁻¹. Antiserum to *R. leguminosarum* 128c53 was prepared as previously described^{9,10}. Micro double diffusion tests were performed as described by Crowle¹¹. The diffusion plates (microscope slides) were prepared with 0.75% Noble agar (Difco) in 10 mM sodium phosphate and 0.85% NaCl, pH 7.0. The diffusion tests were allowed to develop for 48 h at 4°C before being submerged overnight at room temperature in a solution of 10 mM sodium phosphate and 500 mM NaCl, pH 7.0. The plates were then agitated in distilled water for 30 min and stained for 15–30 min in a solution composed of water: isopropanol: acetic acid (13:5:2) containing 0.05% Coomassie brilliant blue R (Sigma). The plates were destained for about 60 min in a solution composed of water-isopropanol-acetic acid (8:1:1).

patterns may be a reflection of lipopolysaccharide structural identity. Second, lipopolysaccharide purified from EXO-1 seems immunochemically identical to lipopolysaccharide isolated from 128c53 *str rif* (Fig. 1). This is particularly pertinent as the lipopolysaccharides of different *Rhizobium* strains are immunologically distinct (R.W.C., C. Napoli, R.E.S. and P.A., in preparation). Finally, the sugar compositions of the carbohydrate moieties of the lipopolysaccharide from EXO-1 and 128c53 *str rif* are very similar as determined by gas chromatography (Table 3).

We have isolated five spontaneous revertants of EXO-1 which have regained the ability to produce copious amounts of extracellular polysaccharide. All five of these revertants have retained resistance to streptomycin and to rifampicin, are sensitive to bacteriophage 1–5, and have regained the ability to nodulate pea seedlings (Tables 1 and 2). In these respects, all five of these revertants seem identical to the starting strain 128c53 *str rif*. It therefore seems likely that a single mutational event is responsible for both the reduced production of extracellular polysaccharide by EXO-1 and the inability of EXO-1 to nodulate pea seedlings.

A simple hypothesis which accounts for the above data is that the reduced production of extracellular carbohydrate by the mutant EXO-1 is related to the inability of this strain to nodulate pea seedlings. Further analysis of EXO-1 and

additional mutants affecting the surface polysaccharides of *Rhizobium* may confirm that, as in other systems^{15,16}, surface polysaccharides participate in the infection of eukaryotes by prokaryotes.

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Mutations to increased antibiotic sensitivity in naturally-occurring gonococci

CLINICAL isolation of mutations giving increased drug sensitivity has not been previously reported. The experiments presented here indicate that a significant proportion of clinical strains of *Neisseria gonorrhoeae* contain mutations which result in markedly increased susceptibility to a variety of antibiotics. Previously, Maness and Sparling¹ isolated and characterised mutants (*env*) of laboratory gonococci which had markedly increased sensitivity ('hypersensitivity') to a variety of antibiotics, detergents and dyes. The *env* mutations were phenotypically dominant over, and totally suppressed, low-level nonspecific drug resistance determined by mutations at a locus formerly designated *ery*², but now designated *mtr*³. Both *mtr* and *env* affected the permeability of the cell envelope⁴. Most of the clinical hypersensitive strains are also shown to possess mutations to nonspecific drug resistance, similar to *mtr*, which are suppressed phenotypically. We have studied strains which included 502 random isolates from patients in North Carolina with uncomplicated gonococcal urethritis and cervicitis, as well as selected erythromycin-hypersensitive strains from London (kindly supplied by I. Phillips), and five penicillinase-producing gonococci (four from the Far East, one from Rotterdam).

Sarubbi *et al.*² have shown that *env* mutants are at least four times more sensitive to erythromycin, fusidic acid, crystal violet (CV) and Triton X-100 (Trx) than are wild-type strains. Accordingly, we determined the minimal inhibitory concentrations (MICs) to erythromycin for all isolates, using an agar dilution method⁵. Wild-type strains, without *env* or *mtr*, generally are inhibited by 0.25–1 μ g ml⁻¹ Ery². Clinical isolates with erythromycin MIC $\leq$ 0.12 μ g ml⁻¹ were considered possible *env* mutants, and were further evaluated for sensitivity to fusidic acid, crystal violet and Triton X-100. Isolates hypersensitive to all drugs were studied for the presence of *env* and suppressed *mtr* mutations by genetic transformation, employing standard recipient strains and methods^{6,7}. A hypersensitive clinical isolate was considered to contain a suppressed *mtr* mutation if it was

capable of donating a gene for nonspecific low-level resistance to erythromycin, fusidic acid and Triton X-100 to wild-type strain FA19⁺.

Clinical *env* mutants were then further characterised as to the type of *env* mutation they possessed by transformation crosses with laboratory strains BR54 (*env*-3) and BR87 (*env*-2). The *env*-2 and *env*-3 mutations are known to occur at separate sites based on recombination frequencies in transformation crosses². To facilitate genetic analysis of the *env*-mutations, high-level rifampin resistant (Rif^R) mutants were selected from the clinical *env* mutants. DNA prepared from such strains were used to transform BR87 and BR54, selecting separately for Rif^R and Env⁺. The Rif^R transformants served as an internal control of the efficiency of the transformation system. The recombination index² (ratio of Env⁺ to Rif^R transformants) was used to determine the proximity of the clinical *env* mutations to *env*-2 and *env*-3.

Results showed that 54 of 347 strains isolated in 1973 (16%) and 30 of 155 strains from 1976 (19%) were hypersensitive to erythromycin (MIC ≤ 0.12 µg ml⁻¹). Of 35 of these strains further evaluated, 32 were hypersensitive to fusidic acid (≤ 0.03 µg ml⁻¹), crystal violet (≤ 2 µg ml⁻¹) and Triton X-100 (≤ 0.25 mg ml⁻¹), whereas none of 53 evaluated wild-type or erythromycin resistant strains were hypersensitive to any of the other drugs; three of five penicillinase-producing gonococci were hypersensitive to erythromycin, fusidic acid, crystal violet and Triton X-100.

Fifteen of 16 tested hypersensitive strains, including the three penicillinase-producing isolates, donated a suppressed gene phenotypically identical to the previously described *mtr* mutation to wild-type FA19. In a typical experiment, a hypersensitive (erythromycin MIC=0.06 µg ml⁻¹) donor transferred to the wild-type (erythromycin MIC=0.25 µg ml⁻¹) recipient a gene for low-level (4.0 µg ml⁻¹) resistance to erythromycin; all Ery^R transformants were also much more resistant to fusidic acid, Triton X-100 and other drugs. Most phenotypically hypersensitive clinical isolates were therefore genotypically resistant.

Transformation crosses between hypersensitive clinical isolates and the isogenic laboratory strains BR54 (*env*-3) and BR87 (*env*-2) confirmed that most of the clinical hypersensitive isolates were *env* mutants (Table 1). In the initial experiments, donor DNA from the clinical isolates was introduced into BR54 and BR87. Results showed that IP6 recombined with much higher frequency with BR87 (*env*-2) than with BR54 (*env*-3), and thus probably contains an *env* mutation very close to *env*-3. FA3024, as well as the penicillinase-producing isolates FA290 and FA362, recombined with high frequency with BR54 but with only very low frequency with BR87. In other experiments, 11 additional hypersensitive strains behaved like FA3024; thus 14 of 16 studied strains were apparently mutant at a site close to *env*-2. In contrast D1519 recombined with both laboratory strains, as well as IP6 and FA3024, and therefore is apparently mutant at a site distinct from those described previously. Reciprocal experiments in which *rif*-2 derivatives of the isogenic set FA19 (*env*⁺), BR54 (*env*-3) and BR87 (*env*-2) were used as donors, and the hypersensitive clinical isolates as recipients, gave very similar results (Table 1).

We suggest that approximately 15% of random clinical isolates from North Carolina, as well as three of five penicillinase-producing isolates, contain *env* mutations, most of which occur in the same gene as the previously characterised *env*-2 mutation, which has recently been shown to result in altered outer membrane proteins by the method of SDS-polyacrylamide gel electrophoresis (D. Walstad, L. Guymon, & P.F.S., unpublished). Presumably the *env* mutation confers on to these strains some selective advantage which cannot be related to their ability to grow in the presence of antibiotics, as these strains are unusually sensi-

Table 1 Recombination indices (Env⁺/Rif^R) in transformations between clinically-isolated antibiotic-hypersensitive (*env*) gonococci and isogenic laboratory mutants containing known *env* mutations*

Donor strains†	Recipient strains‡				
	BR54 (<i>env</i> -3)	BR87 (<i>env</i> -2)	FA3024 (<i>env</i> -5)	FA290 (<i>env</i> -7)	FA362 (<i>env</i> -8)
IP6 <i>rif env</i> -4	0.006	0.4			
FA3024 <i>rif env</i> -5	0.5	0.0005			
FA290 <i>rif env</i> -7	1.2	0.02			
FA362 <i>rif env</i> -8	1.9	0.03			
D1519 <i>rif env</i> -9	0.7	0.2			
FA19 <i>rif env</i> ⁺	2.0	1.5	2.3	1.2	1.5
BR54 <i>rif env</i> -3	< 0.0001	2.3	1.5	0.9	5.0
BR87 <i>rif env</i> -2	1.2	< 0.0001	0.01	0.0009	0.02

*Recombination index equals the number of Env⁺ transformants ml⁻¹ (selected with erythromycin at a concentration of 0.25 µg ml⁻¹) divided by the number of Rif^R transformants ml⁻¹ (selected with rifampin at a concentration of 2 µg ml⁻¹). Results are means of at least two experiments. DNA concentrations were saturating.

†All clinical donor strains (IP6, FA3024, FA290, FA362, D1519) were made rifampin resistant (Rif^R) by purifying spontaneous mutants from plates containing 5 µg ml⁻¹ rifampin. The isogenic laboratory strains (FA19, BR54, BR87) were made Rif^R by introduction of the *rif*-2 allele by transformation.

‡IP6 and D1519 were not competent, and therefore could not be used as recipients.

tive to antibiotics.

In the laboratory these strains autolyse more readily¹ and would, therefore, appear to be at a disadvantage. We have, however, noted that with laboratory strain FA19, the *mtr* mutation consistently results in reduced rates of exponential growth in enriched broth cultures, whereas introduction (by transformation) of an *env* mutation into the derivative carrying *mtr*, FA140, results in partial restoration of normal growth rates *in vitro* (Table 2). Similar results occurred with the clinical *env* strain FA3024 and its *env*⁺ derivative FA507. We have no evidence, of course, that similar growth rates occur *in vivo*, and other explanations such as greater mucosal adherence of *env* mutants are conceivable.

In the pre-antibiotic era, tested strains of *Neisseria gonorrhoeae* possessed neither low-level resistance nor hypersensitivity to antibiotics³. The pressure of antibiotic usage has selected strains with low-level resistance over the past three decades. The commensurate poorer growth characteristics of some of these mutants, though, might have resulted in selective advantage for subsequent mutations which improved growth and decreased resistance. Paradoxically,

Table 2 Log phase doubling times for *env* and *env*⁺ strains

Strain	Ery MIC (µg ml ⁻¹)	Doubling Time (min)†	P Value‡
Isogenic laboratory set*			
FA19 (wild type)	0.25	52.7 ± 4.66	> < 0.005
FA140 (<i>mtr</i>)	4.0	69.3 ± 2.97	> < 0.05
BR87 (<i>mtr, env</i>)	0.06	57.4 ± 5.78	
Isogenic clinical set			
FA3024 (<i>mtr, env</i>)	0.06	49.9 ± 4.27	> < 0.005
FA507 (<i>mtr</i>)	4.0	76.5 ± 6.99	

*Parent strain FA19 and its transformation derivatives have been described by Sarubbi *et al.*². Both FA140 and BR87 contain other mutations besides those shown², but the slow growth of FA140 is due almost entirely to the *mtr* mutation (data not shown). The full genotype of the clinical isolates is not known.

†Mean ± σ. Cells were grown in GC base broth with added supplements³. Growth was at 37 °C in 125 ml side-arm flasks which were agitated in a metabolic shaker with 5% CO₂. The amount of growth was determined by optical density on a Klett-Summerson colorimeter (No. 540 filter).

‡Paired Student's *t* test.

antibiotic usage may be selecting gonococci with both greater antibiotic resistance and, indirectly, greater sensitivity.

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Control of microbial surface-growth by density

IN synchronous cultures of bacteria, the rate of cell elongation¹ or of envelope synthesis² seems to increase abruptly at a particular age. These and other findings suggest³⁻⁵ that bacterial envelope is formed at a constant rate, possibly by enzymes organised in zones, and that some event in the cell cycle leads to a discrete increase in the amount of enzymes or number of zones concerned. (The actual event involved has been variously identified with initiation of chromosome replication⁴, with termination^{1,5} and with the attainment of a critical cell length⁶.) Growth zones are known to occur in the filamentous fungi⁷. The rate at which these zones are able to add new envelope is limited, and since mass increases exponentially⁸, hyphal density eventually begins to rise; when it reaches a critical value, new zones are formed. Thus density (or indeed the concentration of any product synthesised exponentially⁹) could control the rate of surface growth, and we suggest that such is the case in bacteria.

We postulate a constant rate of surface-area synthesis β that doubles once during the bacterial cycle, d min before division¹⁰. For a cylinder with open ends, the cell volume $V(a)$ at age a is given by

$$V(a) = 2^{m-1} R \beta \tau [1 + (a+d)/\tau - m] \text{ for } a \leq (m-1)\tau - d \\ = 2^m R \beta \tau [(a+d)/\tau - m] \text{ for } a \geq (m-1)\tau - d \quad (1)$$

where τ is the mean doubling time of the cell, R is the radius, and $m = [d/\tau]$. If we define ρ_d as the density of the cell at age $\tau-d$, then

$$\rho_d = (M_d/V_d) = (M_i/R\beta\tau)2^{(C+D-d)/\tau} \quad (2)$$

where M_d and V_d are the mass and volume at age $\tau-d$. Here C and D are the times taken to replicate the chromosome and to complete division processes, respectively¹¹, and M_i is the (constant) ratio of cell mass to chromosome origins at the time of initiation of replication¹².

There is reliable experimental evidence that R does not vary very much over the cell cycle during steady-state growth¹³. If we take it to be completely independent of a , then

$$\bar{V} = (M_i/2\rho_d \ln 2)2^{(C+D)/\tau} \quad (3)$$

where $\bar{V}$ has been defined in the usual way as

$$\bar{V} = \int_0^\tau V(a)v(a)da / \int_0^\tau v(a)da,$$

and $v(a)$ is the frequency function of age.

Because ρ_d is considered to be the critical density that leads to the addition of envelope growth-zones, one would expect it to be independent of τ ; $\bar{V}$ should thus vary with τ according to the exponential term. We have tested this prediction against the dimensions of *Escherichia coli* B/r strain H266 growing with a variety of doubling times (Table 1). Non-linear regression analysis to fit equation (3) to average cell volume gave excellent results ($P < 10^{-6}$ from a two-tailed F -test of explained variance). The value obtained for $C+D$ (78.7 ± 7.6 min) is so close to accepted values¹⁴ as to preclude the possibility that ρ_d is exponentially dependent on the growth rate to any great extent. It should be stressed that equation (3) has been derived without any assumptions concerning the dependence of β , R or d on τ . Furthermore, the proportionality of $\bar{V}$ to $2^{(C+D)/\tau}$ found here may also hold true for other Gram-negative bacilli-form bacteria. This follows from the similar dependence of the average mass of *Salmonella typhimurium*^{12,15} on $(C+D)/\tau$ and the independence¹⁶ of the average cell density ρ .

In addition to a maximum at $\tau-d$, cell density goes through a minimum during each cycle. This minimum precedes $\tau-d$ by a fixed fraction of τ , $2-1/\ln 2$ or about 56%, and will thus occur after, at or before cell division according as $d/\tau <, =$ or $> m-1+1/\ln 2$; in all cases, the ratio $\rho_{\min}/\rho_d$ is constant and equal to $\frac{1}{2} \ln 2 \approx 0.94$. (By comparison, ρ/ρ_d , which is also independent of τ , is $2(\ln 2)^2 \approx 0.96$.)

Evidence¹⁷ for just such a variation in ρ over the cell cycle has recently been reported for exponentially growing *E. coli* K12, with measured values of 0.95 for $\rho_{\min}/\rho_d$ and 0.97 for ρ/ρ_d ; $\rho/\rho_{\min}$ was precisely as predicted, 1.020. The occurrence of the maximal density at cell birth and division is consistent with a d of around 44 min; the least-squares estimate provided by the surface-area model (Rosenberger *et al.*, submitted for publication) is 49 ± 4 min. The location of the minimal density, near the middle of the cell cycle, is also as expected.

The time before division at which the rate of envelope synthesis doubles, d , will depend on R and β and the way they vary with τ . The radius of *E. coli* B/r H266 decreases with τ (Table 1); over 90% of the variance ($P < 10^{-6}$) in $R(\tau)$ can be accounted for by the expression $R(\tau) = R_x 2^{\gamma/\tau}$, where $R_x = 0.185 \mu\text{m}$ and $\gamma = 33.6$ min. In order to define the relationship of d to growth rate, it is thus only necessary to determine how β varies with τ . In the absence of definitive data, two simple relationships are considered¹⁰ (see also Rosenberger *et al.*, submitted for publication): no dependence between β and τ , and inverse proportionality. The former predicts d to be a weak function of τ ; the latter to be a constant, $C+D-\gamma$, about 45 min. In either case, and depending on the growth rate, d could coincide with either the initiation or the termination of chromosome replication. This may explain the general lack of agreement in the literature regarding the particular event in the cell cycle considered to be associated with the rate change in envelope synthesis^{1,5,6}.

The dimensions predicted by models postulating linear and exponential rates of envelope synthesis are very similar¹⁸. We would thus like to point out that exponential surface extension between discrete changes in the number of zones can also explain the experimental results, as follows. If the rate of surface growth from a zone increases in proportion to cell mass until the zone reaches its maximum capacity, as is the case in fungi⁷, then cell density will be constant except for the period when all existing zones are acting at full capacity and new ones have not yet become operative; provided this period is short compared to τ , $\bar{V}$ will still be proportional to $2^{(C+D)/\tau}$.

While there seem to be no recognised molecular mechanisms through which changes in density are able to derepress enzyme synthesis, changes in solute concentrations can do so.

Table 1 Mean length $\bar{L}$ and radius R of *E. coli* B/r at various doubling times τ

Medium containing	τ (min)	$\bar{L}$ (μm)	R (μm)
Alanine	160	2.39	0.217
	160	2.20	0.212
	160	2.19	0.212
	124	2.72	0.238
Succinate	105	2.27	0.250
	72	2.64	0.229
Alanine + proline	72	2.80	0.288
	60	2.53	0.232
Glycerol	45	2.62	0.308
	45	2.66	0.300
Glucose	45	2.68	0.312
	32	3.14	0.321
Glucose + casamino acids (Difco)	31	3.35	0.446
	31	3.08	0.406
Glucose + casein	31	3.07	0.417
	31	3.22	0.429
Hydrolysate (Sigma)	24	3.58	0.479
	24	3.29	0.466

E. coli B/r strain H266 was grown in minimal salts medium supplemented with the carbon source indicated¹⁰. Cells, fixed in OsO₄ and agar-filtered, were measured from projections of electron micrographs at a final magnification of 12,000.

If the limiting precursor of an envelope component were synthesised with the same kinetics as is mass, its concentration too would reach a maximum at $\tau - d$; for a relatively large flux and low average concentration, such an increase could be quite substantial.

One should perhaps consider another possibility: that cell radius varies during the cell cycle in such a way as to maintain p constant. Of course, if the cell wall were completely free to adjust to changes in hydrostatic pressure, R rather than p would reach a maximum at $\tau - d$. Here too, the maximum would be only 4% above the average value and 6% above the minimum—variations that are probably beyond experimental resolution¹³. But cell wall, once formed, is able to withstand considerable internal pressures and yields very little (if at all) to forces of expansion. Mitchell¹⁹ measured the phosphate-impermeable volume of stationary *E. coli* as a function of external NaCl concentration in conditions that were later found to cause a substantial cleavage of the peptides crosslinking murein chains²⁰. Nevertheless, less than half the increase in internal osmotic pressure was compensated for by a change in cell volume, the remainder being balanced by the hydrostatic pressure exerted by the cell wall²¹. In principle, in the case of growing cells, new cell wall could attain the necessary magnitude as it is being synthesised in order to maintain constant density despite the rigidity of formed wall. If we assume, as a conservative upper limit, that formed wall can adjust sufficiently to compensate for half the change in volume required to keep p constant, then one can show (and verify easily) that in order for new wall to compensate for the other half, the radius must increase exponentially along the length of the cell from a value of R_0 ($= 2M_0/1n2/\beta\pi$, where M_0 is the mass at birth) at the location corresponding to cell birth to a maximum of $2R_02^{-d/\tau}$ at doubling. There it drops abruptly to half its size and then resumes its exponential climb back to R_0 . Such behaviour is clearly at variance with observation, and we must reject the possibility of constant p and adjustable R .

The applicability of our model will depend in part on the extent to which it can explain the differences in shape observed in closely related strains of *E. coli* growing in a variety of conditions^{5,6,10,14,15,22}, including those produced by specific mutations^{5,23,24}. In this connection, the bizarre alterations of shape found in *E. coli* 15T⁻ growing rapidly in low thymine concentrations²⁵, may be of particular interest.

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Suppression of fever at term of pregnancy

THE newborn human is often unable to develop a fever following exposure to various infectious agents^{1–3} and newborn lambs are similar, in that they do not develop a fever in response to a bacterial endotoxin challenge in the first few days of life⁴. Lack of the febrile response is thought to be due to the need for a process of 'sensitisation' of the lambs, or to the immaturity of some aspect of the fever-production process^{5,6}. Newborn guinea pigs and rabbits also, do not become febrile or show a diminished response to a challenge with endotoxin during early postnatal life^{7,8}. We have examined the response of the pregnant ewe to pyrogens at and near the time of birth, for although they become febrile during pregnancy⁹, their response to endotoxin at term has not been studied. We report here that the febrile response induced by a bacterial endotoxin or by endogenous pyrogen is suppressed in the ewe for a period extending from 2 to 5 d prepartum to several hours postpartum.

Suffolk, Dorset and cross-bred ewes were studied at various times ranging from 8 d prepartum to 60 h postpartum. The ewes were injected by way of the jugular vein with 30 μg of bacterial pyrogen derived from *Salmonella abortus equi* (SAEP) in a 3-ml volume of sterile physiological saline. This quantity of SAEP gave a fever of 1.3 ± 0.15 °C in non-pregnant adult sheep. The SAEP was administered to each ewe at 3-d intervals, in order to avoid the production of refractoriness to the pyrogen. Temperatures were measured continuously by a thermistor probe inserted about

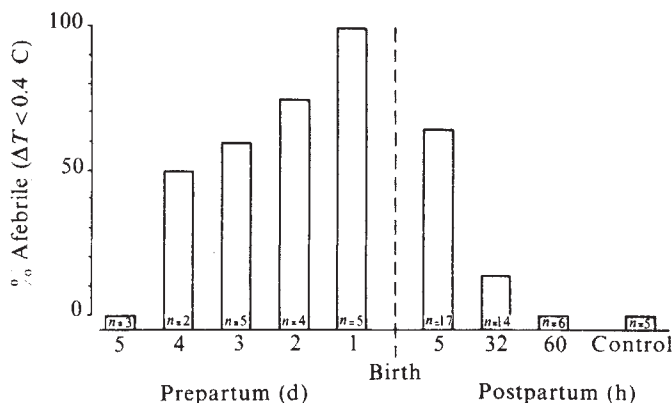


Fig. 1 The proportion of ewes not responding with a fever following 30 µg SAE pyrogen given intravenously is represented by the vertical bars. The animals were injected from times ranging from 5 d prepartum to 60 h postpartum. The responses of non-pregnant adult ewes is represented on the far right. The broken vertical line represents the time of birth.

20 cm beyond the anal sphincter and the output of the thermistor was displayed on a recorder. Rectal temperature was monitored until it had remained steady for 30 min before injection and then was monitored for 200 min after injection. A fever was considered to be a 0.4 °C or greater rise in temperature above that at the time of injection.

Endogenous or leukocyte pyrogen (EP) was prepared from sheep granulocytes, obtained by a sterile peritonitis induced with 3% starch solutions after the method of Fessler *et al.*¹⁰. This EP was found to be endotoxin-free using Murphy's rabbit test¹¹ and by the Limulus assay for Gram-negative material^{12,13}. A fever with a maximum of 1.85 ± 0.10 °C was produced by 0.2 ml of this EP in the non-pregnant adult sheep. The EP was injected in the same manner as was the SAEP after it was found that the ewes did not respond to SAEP with a fever. This procedure was intended to determine at which step in the sequence of events of fever production the inhibition was manifest, since EP is thought to be released from cells of the reticuloendothelial system once they are stimulated by the endotoxin or antibody-endotoxin complex. It is thought that EP stimulates some higher centre(s) which react by driving heat production and heat conservation.

The ewes responded with fever to SAEP until a time which varied between 4 and 2 d prepartum. As shown in Fig. 1, 5 d before parturition, all the three ewes tested developed a fever similar to that in the non-pregnant controls. At 3 and 4 d before parturition four of seven ewes were afebrile following the injection of pyrogen. At days 1 and 2 prepartum, eight of the nine ewes tested failed to develop a fever. The ewes were injected with SAEP 5 h postpartum, and 11 of 17 remained afebrile, whereas at 32 h postpartum, 12 of 14 ewes could again become febrile. By 60 h, all the six ewes tested were febrile. Most EP challenges fell within the 0–48 h prepartum period, as they were given after the ewe became afebrile to SAEP. Although the number of experiments using EP is small, it was observed that four of seven ewes, 1 and 2 d prepartum responded to EP with fevers whereas three were afebrile. It was not possible during this breeding season to do additional experiments with EP at other times. All control animals responded with fever following every injection of both SAEP and EP.

These data suggest the presence of some naturally occurring fever-suppressing system. First, there could be a humoral antipyretic agent which is shared by the circulation of both ewe and foetus. This is compatible with the observation that the newborn lamb also does not become febrile to either SAEP or EP. Second, it could be possible that the ewe is afebrile because of a circulating antipyretic but the newborn lamb, on the other hand, is afebrile as the result of some immature cellular system in the fever pathway. The fact that the EP never produced a fever in the newborn animal suggests that it may be afebrile by some other mechanism or, consistent with the first possibility, that the titre of antipyretic

substance is much higher than in the ewe. Injections of EP were given in order to determine at which step in the sequence of events inhibition is occurring within the system. The differential response to the EP among the prepartum ewes, four febrile and three afebrile, at 0–48 h before birth, suggests that the inhibition occurred after EP had been elaborated^{11–14}. An explanation consistent with this is that with our dose of EP, the titre was higher than that naturally produced by the reticuloendothelial system in response to 30 µg SAEP. Since EP produced a fever in four of seven animals, it is likely the inhibition was at some stage after the elaboration of EP and raises the possibility that higher doses of EP may overcome this inhibition. This explanation requires that inhibition is not total but that the putative antipyretic acts by competitive inhibition of EP or some later mediator of fever.

The evidence presented here that there is a suppression of fever near term, would indicate some adverse consequences of fever at term. The ewes which produced fevers to EP during their critical period, however, showed no more trouble with parturition than the afebrile mothers. The lambs of EP febrile mothers were no less viable than those of EP afebrile mothers, judging from observations such as ability to suckle soon after birth, strength and growth rate.

It remains to be seen if term ewes can become febrile to any pyrogenic agent other than EP or if any other mammals, particularly human, show in some way this endogenous antipyretic phenomenon. The identity of such an antipyretic is another critical question and its characterisation could add significantly to our understanding of the febrile process and antipyresis.

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Action of *Corynebacterium ovis* exotoxin on endothelial cells of blood vessels

Corynebacterium ovis (*C. pseudotuberculosis*) causes suppurative infections of sheep, goats and horses and occasional infections in other species². Like *C. diphtheriae*, it produces a powerful exotoxin *in vitro*. But unlike the situation with *C. diphtheriae*, there is no evidence of acute or chronic intoxication, while the lesions are characteristically pyogenic with suppuration and abscess formation at the initial site of infection (usually a skin wound). This is followed frequently by secondary abscess formation in regional lymph nodes and sometimes internal organs. Toxin is produced *in vivo* as well as *in vitro* because circulating antitoxin is found in infected animals. The major role of *C. ovis*

toxin in natural infection seems to be facilitation of the spread of the causative bacteria by its action as a permeability factor. It thus causes marked leakage of plasma from small blood vessels at the site of infection which floods lymphatic spaces and increases the risk of bacteria being carried by lymphatic drainage from the site of infection to regional lymph nodes^{3,4}. We now report experimental evidence that *C. ovis* toxin is a phospholipase D which attacks the sphingomyelin of endothelial cells of blood vessels.

We considered two possible modes of action for *C. ovis* toxin. The first was an indirect effect in which toxin acts on mast cells closely associated with blood vessels, leading to liberation of histamine and 5-hydroxytryptamine which then act on adjacent endothelial lining membranes of blood vessels, increasing their permeability. The second was a direct action of the toxin molecule on endothelial cells. When mast cells from the peritoneal cavity of rats and mice were exposed to toxin, no degranulation was observed. This accorded with the observations of Smith and Miles⁵ who found no evidence of liberation of histamine or 5-hydroxytryptamine when *C. ovis* culture was introduced into the peritoneal cavity of the rat.

Souček *et al.*^{6,7} have reported that purified *C. ovis* toxin is a phospholipase D which splits sphingomyelin into *N*-acyl-sphingosyl (ceramide) phosphate and choline. This action occurred both when purified sphingomyelin was used as substrate and when the lipid was incorporated in the lipoprotein of the cell membrane of erythrocytes. This suggested that *C. ovis* toxin acts in a similar manner on the sphingomyelin of endothelial cells lining blood vessels. We have examined this by exposing living endothelial cells lining the aorta of sheep and rabbits to purified *C. ovis* toxin. We then examined chloroform: methanol extracts of the treated cells for evidence of ceramide phosphate and cholin—the breakdown products of sphingomyelin by a phospholipase D. Washed sheep and rabbit erythrocytes were also exposed to toxin for comparison.

Toxin was precipitated from culture filtrates by 35–65% saturation with ammonium sulphate. This was followed by ion-exchange chromatography on a column of Sephadex CM 50, pressure dialysis and freeze drying. (Further purification has recently been achieved by use of DEAE columns, but the biological properties have remained essentially the same. Details will be published elsewhere.) A solution of 1 mg ml⁻¹ in phosphate-buffered saline (PBS) had a minimal reactive dose in the rabbit of 0.2 ml of 10⁻⁵ dilution. The aortae of five sheep and two rabbits were exposed to toxin (1 mg ml⁻¹ in phosphate-buffered saline containing 4 mM MgCl₂); and as controls, aortae of two sheep and one rabbit were similarly treated either with toxin inactivated by heating at 60 °C for 2 h, or buffer only. Animals were anaesthetised and most received heparin intravenously to prevent clotting. After bleeding out, the abdomen and thorax were quickly opened, cannulae were inserted into the aortic arch and near the bifurcation of the abdominal aorta; the aorta was then perfused with PBS while all arterial branches were ligated and severed. The aorta was removed, filled with toxin or control fluid, suspended in PBS and held at 37 °C for 1–2 h. Phospholipids of the endothelial lining were then extracted by chloroform: methanol (2:1 v/v) based on the method described by Dawson *et al.*⁸. Erythrocytes from 50 ml of heparinised blood were washed three times in PBS containing 4 mM MgCl₂. One-half of the deposited cells were mixed with toxin (1 mg ml⁻¹ and the other with heat-inactivated toxin. Suspensions were incubated at 37 °C, and cells were finally extracted with chloroform: methanol (2:1).

Equal volumes (200 µl for aortae, 150 µl for erythrocytes) of control and treated extracts were spotted on to thin-layer chromatography (TLC) plates (Merck silica gel 60F-254). Sphingomyelin and ceramide phosphate were included as markers. TLC plates were developed with chloroform-methanol-water-acetic acid (65:50:4:1 by volume). The phospholipids were detected by a phosphorus-specific spray⁹. Some TLC plates were sprayed with a choline-detecting reagent¹⁰.

In all extracts of toxin-treated aortae and erythrocytes of both

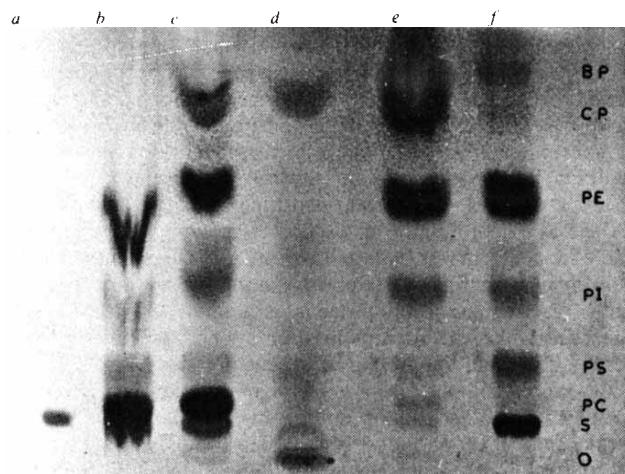


Fig. 1 TLC plate extracts of toxin-treated and control aortic endothelium and erythrocytes of sheep. Vertical lanes: a, sphingomyelin; b, control aortic extract; c, toxin-treated aortic extract; d, synthesised ceramide phosphate; e, toxin-treated erythrocytes extract; f, control erythrocytes extract. Horizontal lanes: BP, blood pigments; CP, ceramide phosphate; PE, phosphatidyl ethanolamine; PI, phosphatidylinositol; PS, phosphatidyl serine; S, sphingomyelin; O, origin.

sheep and rabbits, sphingomyelin was degraded to ceramide phosphate and choline. Figure 1 shows a TLC plate of the sheep extracts sprayed for phospholipids.

After completion of these experiments our attention was drawn to reports that certain enzymes, including phospholipases, may not be inactivated by chloroform: methanol used to extract tissues. If this were the case with *C. ovis* toxin, then the ceramide phosphate demonstrated in our extracts of aortic endothelial cells and erythrocytes may not have resulted from direct action of toxin on sphingomyelin bound to protein in the endothelial cell membrane, but may have occurred as the result of phospholipid degradation by still active toxin after extraction by organic solvent. We therefore set up the following tests. (1) We mixed 0.5 ml of emulsion of sphingomyelin in water with 0.5 ml of toxin (1 mg ml⁻¹ in phosphate-buffered saline + MgCl₂); added 20 ml of chloroform: methanol (2:1 v/v), incubated them together for 2 h at 37 °C and ran the mixture on TLC plate. (2) As for (1) but the mixture was tested on a TLC plate immediately after mixing. (3) and (4) as for (1) and (2) but a suspension of washed sheep erythrocytes was substituted for sphingomyelin emulsion. In each case phospholipase D activity of *C. ovis* toxin was annulled by exposure to the chloroform: methanol, even for a few minutes.

We have thus confirmed (the evidence of Souček *et al.*^{6,7}) that *C. ovis* toxin is a sphingomyelinase which splits the sphingomyelin of the cell membrane of erythrocytes into ceramide phosphate and choline, but we have extended our experimental observations to show that the toxin has a similar action on the sphingomyelin of the vascular endothelial membrane. This raises the question of the possible significance of this enzyme action of *C. ovis* toxin in relation to its permeability effect on blood vessels.

It is interesting that *C. ovis* toxin produces its permeability changes rapidly within minutes. This seems to exclude the possibility that this early action was due to permeability factors derived from leukocytes or platelets which are not seen to adhere to the luminal surface of endothelial cells until 30 min or longer after injection of toxin.

In spite of extensive study, little is known of the biochemical and biophysical causes of increased vascular permeability which is such a striking feature of acute inflammation and the action of certain bacterial toxins^{11–14}.

Majno *et al.*^{15, 16} have shown that exposure of small blood vessels to the well-known permeability agents, histamine and 5-hydroxytryptamine, leads to partial disconnection between

endothelial cells along intercellular junctions, thus forming gaps through which leakage of plasma occurs. Among possible factors leading to the formation of such gaps may be the chemical breakdown of specific junction constituents (for example, the protein connexin isolated by Goodenough¹⁷ from junctions between mouse liver cells), and/or increased tension on junctions resulting from contraction of the cell membrane caused by chemical changes in important constituents over the whole cell surface. Sphingomyelin, which is broken down by *C. ovis*, forms part of an important structural lipoprotein of the cell membrane. It is interesting that the alpha toxin of *Clostridium welchii*, another active permeability agent, is a phospholipase C and attacks lecithin, the phospholipid component of another important structural lipoprotein of cell membranes¹⁸.

We are examining the possible significance of the enzyme action of *C. ovis* toxin on sphingomyelin in relation to its permeability effect on blood vessels.

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Differential cell adhesion may result from nonspecific interactions between cell surface glycoproteins

I SUGGEST that if different cell types have different glycoprotein coatings they will tend to adhere to each other with different intensities, and sort out into separate tissues simply as a result of weak nonspecific interactions between the glycoproteins without any special mechanisms such as binding by specific recognition proteins. This could be responsible for many, but certainly not all, cell recognition and adhesion phenomena (reviewed in refs 1 and 2).

Cells are coated with glycoprotein and glycolipid so they would seem from the outside like giant macromolecules of glycoprotein (for simplicity, the glycolipid can be pictured as providing extra oligosaccharide chains in this glycoprotein) and they ought to show behaviour analogous to the behaviour of large polymers in solution.

Large polymers when mixed in solution tend to undergo incompatible phase separation, the solution separating into immiscible phases each containing predominantly one polymer

species³. For example, a sufficiently concentrated solution of polyethylene glycol and dextran separates into two phases, and many other polymers, when added to the system, will form further separate phases. Two separate phases can even form from solutions of one polymer and a small molecule, for example polypropylene glycol and potassium phosphate or glucose³. The behaviour of small molecules in solution is usually determined mainly by the entropy of dilution, representing random distribution of the solute throughout the solvent. For polymers the entropy of dilution remains, to a first approximation, the same per molecule but the enthalpy of interactions between the polymer molecules increases in proportion to the size of the molecules and at sufficiently high molecular weight may become the dominant influence. For hydrophilic polymers these interactions will mainly be weak and non-specific, such as hydrogen-bonding, van der Waal's, fixed dipole-dipole and hydrophobic interactions. Such interactions are generally stronger between like molecules than between unlike molecules, so large polymers tend to segregate into separate phases⁴. (There are, of course, many polymers, particularly charged ones, that do interact with each other more strongly than with themselves. They may form complexes that separate as a single phase from solution leaving a polymer-free phase.) An additional factor promoting segregation of unlike molecules arises from steric effects. Each polymer molecule excludes other molecules from a volume of solution often much larger than its physical volume, particularly if it is extended rather than globular⁴. At sufficiently high concentration the freedom of polymer molecules to pack together, represented by an entropy term, becomes a significant factor favouring the segregation of polymer species of different molecular size. (For further discussion and thermodynamic treatment see refs 4 and 5.) So mixed solutions of hydrophilic polymers tend to show phase separation, and the larger the molecules the greater the tendency and the weaker the interactions needed to cause phase separation. It has already been pointed out that proteoglycans and glycoproteins ought to show this behaviour⁶.

If cells of different types have different glycoprotein coats they would in general be expected to form separate phases, that is, to sort out into aggregates of a single type of cell. If they are suspended in a medium such as plasma, containing small polymers, or even just saline, one phase might be cell-free, in other words cells of all types would aggregate, then sort out within the aggregate. (This, incidentally, may have something to do with the need for serum components in culture media for cells⁷.) It is well known that phase separation is a good description of the sorting out of embryonic cells. To quote Moyer and Steinberg⁸ "rearrangements [of cell aggregates] . . . resemble in striking detail the rearrangements of immiscible liquids", cells corresponding to molecules of the liquids.

The energy released by phase separation compares favourably with the energy needed to bind two cells together. Agglutination of two red cells requires less than 10^3 antibody molecules⁹ which would give about 10^3 kcal of binding energy per mol of cells. When, for example, two like cells come into contact over an area of $50 \mu\text{m}^2$, there is a loss of $100 \mu\text{m}^2$ of interface between the cells and either medium or unlike cells. For these cells to adhere as strongly as the red cells the interfacial free energy released by the contact would have to be only 10^3 kcal per $6 \times 10^{23} \times 100 \mu\text{m}^2$ or 7×10^{-5} erg cm^{-2} , much less than the 10^{-3} to 10^{-1} erg cm^{-2} typical of the examples of phase separation above³. It corresponds to only 2×10^{-3} kcal per mol of surface glycoprotein if the cell surfaces have the same surface density of integral membrane glycoproteins as the red cell¹⁰, 10^6 per $100 \mu\text{m}^2$.

So merely by carrying different glycoprotein coats cells might well be expected to show differential adhesion phenomena. For example, in development different cell types would form separate phases, that is, tissues. Less obvious examples could be the binding of desialylated serum glycoproteins to liver cell surfaces, though this may be mediated by a true lectin activity¹¹,

and the selective penetration of capillary walls by leucocytes, where the capillary wall can be viewed as a phase into which leucocytes can reversibly partition, but which is immiscible with other components of blood.

This explanation of apparently specific cell interactions in terms of large numbers of weak nonspecific forces conflicts particularly with the widely held view that cells interact through specific binding by recognition proteins with lock-and-key active sites^{1,2,11}. Such proteins probably do play a part in some mechanisms^{1,2,11}; the point is that they may not be necessary. Several authors¹ have previously suggested that the forces between cells are nonspecific; in particular, Steinberg¹² showed some years ago that nonspecific forces could produce immiscibility of aggregates of different types of cell. One way in which the consequences of the view presented here may differ from the consequences of the lock-and-key view is that immiscibility of dissimilar glycoprotein coats on different cell types can provide a repulsive interaction in the sense that active cell migration through an incompatible phase would be resisted. In contrast the simple lock-and-key picture tends to predict positive or zero adhesion.

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Hybrid cell lines secreting monoclonal antibody specific for major histocompatibility antigens of the mouse

HYBRIDISATION of a myeloma cell line with hyperimmunised spleen cells can provide hybrid cell lines secreting monoclonal antibodies of predefined specificity^{1,2}. Lines have thus been established which secrete homogeneous antibodies against antigens such as sheep red blood cells¹, the hapten 2,4,6-trinitrophenyl², influenza virus³, horse red blood cells, the haptens (4-hydroxy-3-nitrophenyl)acetyl and (4-hydroxy-5-iodo-3-nitrophenyl)acetyl, group A streptococcal carbohydrate, hen egg lysozyme, chicken γ globulin, the synthetic polypeptide poly-L-(Tyr, Glu)-poly-D,L-Ala-poly-L-Lys⁴ and antigens encoded by the major histocompatibility complex (MHC) of the rat⁵. We report here the derivation of three cloned hybrid cell lines which synthesise antibodies detecting different public antigenic specificities of the mouse H-2 complex. These hybrid cell lines can be grown in the mouse as ascites tumours, and the ascites fluids obtained contain large quantities of homogeneous anti-H-2 antibodies.

BALB/c (H-2^d) spleen cells hyperimmunised with CBA (H-2^k) lymphocytes were fused with the mouse myeloma P3-X63-Ag8 using polyethylene glycol as a fusion reagent (see

Fig. 1 legend). Culture supernatants of 200 individual hybrid cell lines obtained in one fusion experiment were screened for anti-H-2 activity by a complement dependent cytotoxicity assay or a cellular radioimmunoassay in which antibody bound to target cells was monitored by uptake of ¹²⁵I-labelled staphylococcal protein A (¹²⁵I-SpA)⁷. Five of the 200 supernates were cytotoxic to CBA but not BALB/c target cells. Their protein A assay response indicated that the secreted antibodies belonged to the IgG class⁸ (Fig. 1). No hybrid cell line was active in only one assay.

The hybrid cell lines were cloned in soft agar and three individual clones, one of each hybrid, with activity against CBA target spleen cells, were selected for further characterisation of their products. These clones, 5R4, 27R9 and 30R3, were injected intraperitoneally into Pristan-pretreated BALB/c mice. The hybrid cell antibodies were purified from ascites fluid by agarose block electrophoresis. Spectrophotometric determination of the protein concentration in different fractions revealed a single peak in the γ globulin region close to the starting point. The amount of purified protein in this peak was calculated from the optical density at 280 nm, assuming an extinction coefficient E (1 cm pathlength) of 1.4 for a solution containing 1 mg protein per ml. The yields were 40 mg from 4 ml ascites fluid for antibody 5R4, 45 mg from 3 ml ascites fluid for antibody 27R9, and 45 mg from 3 ml ascites fluid for antibody 30R3. Microzone electrophoresis and isoelectricfocussing demonstrated that the protein peak contained both the P3-X63-Ag8 myeloma protein MOPC 21 (γ 1, κ) (refs 1, 12) and additional protein components, presumably representing the cytotoxic antibody and hybrid molecules¹². Most of the antibody activity was found in the fractions with the highest protein content. These were analysed in the Ouchterlony

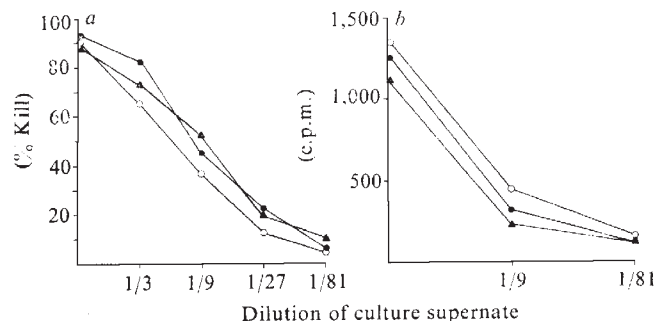


Fig. 1 BALB/c (H-2^d) mice were primed by skin grafting against CBA (H-2^k) allo-antigens and hyperimmunised by three intraperitoneal injections of $1-3 \times 10^7$ CBA spleen cells in 10-d intervals beginning with the first injection 2 weeks after priming. Five days after the last injection, cells from four BALB/c spleens were fused with the myeloma P3-X63-Ag8. Cells were fused employing a modification¹⁴ of the method described by Pontecorvo *et al.*¹⁵, except that polyethylene glycol with a molecular weight of 4,000 was employed and the ratio of spleen to myeloma cells was 2:1. After fusion, the cells were distributed in multiwell tissue culture plates (Costar No. 3524) at a density of 2×10^6 spleen cells per well and cultured in HAT medium¹. Two weeks later, cell growth became microscopically evident in 200 out of 240 wells. Culture supernatants of these positive wells were assayed for anti-H-2 activity either by a two step microcytotoxicity assay using rabbit serum as a source of complement (a gift from Dr J. Jensenius, Copenhagen) or by a cellular radioimmune assay in which antibody bound to target cells is monitored by subsequent uptake of ¹²⁵I-labelled staphylococcal protein A which binds exclusively to immunoglobulin of the IgG class⁹. We followed the method described by Dorval *et al.*⁷. The titration curves for (a) cytotoxicity and (b) ¹²⁵I-binding properties of culture supernatants of hybrid cell lines 5 (●), 27 (○) and 30 (▲) are shown. Five out of 200 hybrid cell lines in this particular fusion experiment secreted anti-H-2 antibodies. One of the five hybrids lost its activity and another was lost by bacterial infection. In other fusion experiments, more than 1,000 hybrid cell lines were screened for anti-H-2 activity, but no positive lines were detected. At present we cannot define the precise experimental conditions to reproduce the successful hybridisation.

technique using class and subclass specific antisera (obtained from B. Liesegang & A. Radbruch, Cologne, and Meloy Co.). None of the three hybrid antibodies reacted with anti- μ and anti- α antisera. In all cases, precipitin lines appeared with anti- γ 1 antisera, while antibody 30R3 reacted with anti- γ 2b, and antibodies 5R4 and 27R9 with anti- γ 2a. No reaction was found with anti- λ sera. This suggested that all preparations contain MOPC 21 chains as already indicated by microzone electrophoresis and isoelectric focussing, and that antibody 30R3 carries γ 2b and antibodies 5R4 and 27R9 γ 2a heavy chains. All three antibodies probably possess κ light chains.

Ascites fluids containing antibodies 5R4, 27R9 or 30R3 were tested on different H-2 haplotypes and intra-H-2 recombinant haplotypes to determine the antigenic specificities detected by these monoclonal antibodies (Table 1). The antibodies secreted by the three clones exhibited strong reactivity on CBA (H-2^k) but did not react with BALB/c (H-2^d) or DBA/2J (H-2^d) spleen cells. To determine whether H-2 specificities are detected by the antibodies, they were tested on B10.BR (H-2^k) and B10.D2 (H-2^d) target cells, which share the B10 background and differ only in their H-2 haplotypes. All three antibodies reacted with B10.BR (H-2^k) but not with B10.D2 (H-2^d), and thus can detect specificities of the H-2^k haplotype.

Various intra-H-2 recombinant strains were used to evaluate detection of the specificities of the K, I and D regions of the H-2 complex. All three antibodies reacted strongly with B10.A (H-2^k) target cells which have the K end of H-2^k and the D end of H-2^d. Antibody 5R4 did not react with C3H.OH (H-2^o) cells which carry H-2^k antigens of the D region, while antibodies 27R9 and 30R3 occasionally showed weak reactions with these cells at high concentrations. Antibodies 5R4 and 27R9 did not react with the intra-H-2 recombinant A.TL (H-2^u) or with A.SW (H-2^s), while antibody 30R3 showed reactivity with both strains. This indicates that antibodies 5R4 and 27R9 do not detect Ia antigens of the H-2^k haplotype and that the corresponding antigens are also not present in the K region of the H-2^s haplotype. It further suggests that antibody 30R3 also does not react with Ia antigens but crossreacts with a specificity of the K region of H-2^s. Another strong argument against the detection of Ia determinants by these antibodies is

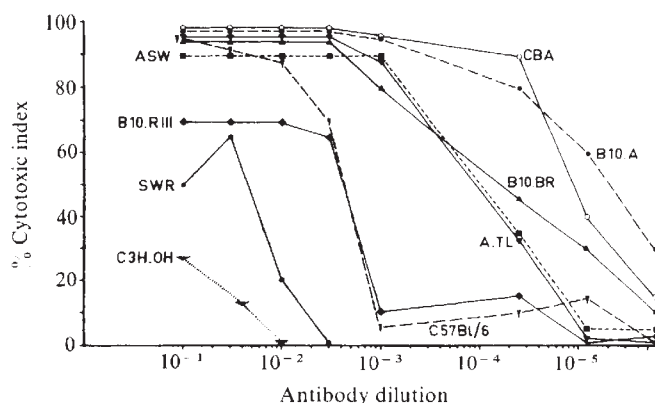


Fig. 2 Cytotoxic activity of antibody 30R3 on spleen target cells of different H-2 haplotypes. Ascites fluid of tumour-bearing BALB/c mice was used as the source of antibody 30R3. As the data were obtained in different assays, the cytotoxicity values are presented as cytotoxic indices (see legend to Table 1).

the fact that they kill close to 100% of spleen cells. This has not been observed with anti-Ia antisera since Ia antigens are predominantly expressed on B lymphocytes⁹. These data therefore suggest that all three antibodies detect determinants encoded by the K region of the H-2 complex.

Further experiments demonstrated that antibodies 5R4 and 27R9 showed no reactivity on C57BL/6 (H-2^b), while antibody 30R3 showed 100 to 1,000-fold lower cytotoxic activity than on CBA (H-2^k) or B10.BR (H-2^k) cells. All three antibodies reacted with antigenic specificities also present on B10.RIII cells which carry the H-2^k haplotype. Antibodies 5R4 and 30R3 but not 27R9 also displayed activity on SWR/J (H-2^a) cells. Comparison of these results with the distribution of antigenic specificities of the K region of the H-2 chart⁶ suggests that antibody 5R4 may detect specificity H-2.11, antibody 27R9 specificity H-2.25 and antibody 30R3 H-2.5. This was confirmed (Table 1) when all three antibodies were entirely negative when tested on A.CA (H-2^f) cells, a haplotype which expresses none of the H-2 specificities, 5, 11 and 25.

The differences in reactivities of antibodies 5R4 and 27R9 observed on target cells carrying different haplotypes may be due to experimental variations and do not necessarily reveal a polymorphism of the corresponding antigenic determinants. Antibody 30R3, however, reacted equally strongly with H-2^k and H-2^s cells, showed weak crossreactivity with haplotypes H-2^r and H-2^a and very weak reactions with H-2^o target cells (Fig. 2). However, all these haplotypes express specificity H-2.5 encoded either by the K region (H-2^{b,k,s,r,a}) or by the D region (H-2^o) of H-2 (ref. 6). It has already been suggested by conventional H-2 serology that specificity H-2.5 encoded by different H-2 haplotypes reflects non-identical but crossreactive antigenic determinants^{6,10,11}. Assuming that antibody 30R3 really detects specificity H-2.5, the titre differences observed in the reactions of this antibody with target cells from different haplotypes (Table 1, Fig. 2) seem larger than titre differences with conventional anti-H-2 antisera, which have relatively low titres. This may be due to the different strain combinations used for raising conventional antisera⁶ and for the production of antibody 30R3. In addition, even if the same strain combination had been used for the production of the hybrid cell antibody, one might still expect the fine specificity of this monoclonal antibody to be different from that of a complex antiserum. It is indeed striking to see that the antigenic specificities detected by our three hybrid cell antibodies correlate largely with antigenic specificities enlisted in the H-2 chart⁶. The exquisite specificity of serological typing reaction thus seems to be fully retained at the level of monoclonal antibodies. This finding is important¹², and suggests that monoclonal antibodies will play a key role in future histocompatibility typing.

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Table 1 Cytotoxicity of BALB/c anti-CBA (H-2^d anti-H-2^k) monoclonal antibodies obtained from ascites fluids of tumour bearing mice

Strain	H-2 Haplotype	H-2 Complex			Cytotoxic titre of antibody*		
		K	I	D	No. 5R4	No. 27R9	No. 30R3
CBA	k	k	k	k	90,000	40,000	100,000
BALB/c	d	d	d	d	—	—	—
DBA/2	d	d	d	d	—	—	—
B10.BR	k	k	k	k	25,000	20,000	20,000
B10.D2	d	d	d	d	—	—	—
B10.A	a	k	k/d	d	25,000	20,000	200,000
C3H.OH	o	d	d	k	—	—†	—†
A.TL	tl	s	k	d	—	—	9,000
C57BL/6	b	b	b	b	—	—	400
A.SW	s	s	s	s	—	—	10,000
B10.RIII	r	r	r	r	25,000	100,000	400
SWR	q	q	q	q	4,000	—	50
A.CA	f	f	f	f	—	—	—

*Reciprocal antibody dilutions at which 50% killing of target spleen cells of different H-2 haplotypes were obtained. The data are corrected for background killing as follows:

$$\text{cytotoxic index (\%)} = \frac{\% \text{ dead cells with antisera} - \% \text{ dead cells control}}{100 - \% \text{ dead cells control}} \times 100$$

The 50% values are taken from complete titration curves an example of which is presented in Fig. 2.

†In some tests cytotoxic activity was observed at antibody concentrations ≥ 10 .

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Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus

CELL mediated immune reactions in mice involving cytotoxic thymus derived lymphocytes have two important features; they are antigen-specific¹ and the structures coded for by the major murine histocompatibility gene complex (*H-2*) play an important part in killer cell-target cell interaction¹. This finding is not unique to mice since it has also been reported for rats, humans and chickens^{2–4}. Two hypotheses have been suggested to explain this dual specificity of T killer cells; one proposes that T killer cells possess two separate structures, one of which recognises self *H-2* structures and the other a non-*H-2* antigen (dual recognition). The other hypothesis proposes that T killer cells possess one receptor which recognises an *H-2* antigen modified by a non-*H-2* antigen (modified self)^{1,5}. Evidence to test these hypotheses was sought by preparing chimaeras made by reconstituting lethally irradiated *F*₁ mice with bone marrow stem cells of one parent (Parent→*F*) refs 6–8) or neonatally tolerant mice⁹ (mice of a parental *H-2* type injected with *F*₁ cells as neonates). As a result, Parent → *F*₁ chimaeric lymphocytes lysed

infected and trinitrophenyl (TNP)-modified targets of both parental *H-2* types, suggesting that *H-2* antigens of the killer cells were not involved in the lytic interaction. In contrast, lymphocytes from neonatally tolerant mice did not lyse infected targets from the incompatible parent⁹. Although these results can be explained by postulating a single receptor, they also fit the dual recognition hypothesis^{6–10}. Bevan has shown that irradiated bone marrow chimaeras of the type (*A* × *B*)*F*₁ → Parental *A* recipient generated preferentially cytotoxic T cells against minor histocompatibility antigens in association with the *H-2* haplotype of the recipient *A*; he concluded that the chimaeric host determines the specificity of T cell restriction either through a thymic selection process as proposed by Jerne or alternatively through an antigen presentation mechanism.

In similar experiments with *F*₁ → Parent chimaeras, we found that tolerance alone was again not sufficient to generate both virus-specific and TNP-specific cytotoxic T cells against antigen in association with the tolerated *H-2*. These results and studies on the role of the thymus in the differentiation of *H-2* restricted killer cells seem to indicate that precursor T cells from lymphohaemopoietic stem cells are selected to recognise the *H-2* markers during their differentiation in the thymus; this learning process is independent of the T cell's own *H-2* haplotype¹⁶.

*F*₁ hybrid → Parent chimaeras were formed by irradiating parental mice with 925–950 rad and then reconstituting them with *F*₁ bone marrow cells⁷. The chimaeras were infected 10–12 weeks later with vaccinia virus [10⁷ plaque forming units (PFU) per recipient] and killed 6 d later. Spleen cells from each individual were typed serologically for *H-2*, after which virus-specific cytotoxicity was assessed in a ⁵¹Cr release assay on infected and uninfected target cells (Table 1). Spleen cells from other *F*₁ → Parent chimaeras were also typed for *H-2* and then sensitised *in vitro* against TNP-modified stimulator cells (Table 2). The serological results show that the lymphocytes of the *F*₁ → Parent chimaeras were all of *F*₁ type. However, cytotoxic activity was directed exclusively against infected or TNP-modified targets of the recipient parental type but not against targets from the second parent. There was no activity against normal targets of both parental *H-2* types either *in vitro* or *in vivo*. Thus, cytotoxicity was found only against targets that were *H-2* identical with *F*₁ donor and parental chimaera recipient, but not against targets of the second parental *H-2* type, despite the inherent tolerance of *F*₁ lymphocytes for both parental antigens and the presence of large amounts of infected or TNP modified cells expressing all relevant *H-2* antigens. Other chimaeras were constructed of the type (*A* × *B*)*F*₁ → (*A* × *C*)*F*₁. When lymphocytes from such chimaeras were tested in the TNP system and sensitised against TNP (*B* × *C*)*F*₁ cells, strong TNP-specific cytotoxicity was generated against C-TNP but none against B-

Table 1 Vaccinia-virus specific cytotoxic activity generated in *F*₁ → Parent irradiation bone marrow chimaeras *in vivo*

Immune lymphocytes from vaccinia infected mice* (<i>H-2</i> type)	Lymphocyte to target cell ratio	% Specific ⁵¹ Cr release from target cells			
		<i>L</i> (<i>k</i>)		<i>D2.GD</i> (<i>d</i> / <i>b</i>)	
		Vaccinia treated	Normal	Vaccinia treated	Normal
Bone marrow chimaeras					
BALB/c × <i>A</i> → <i>A</i>	40:1	81†‡	0	5	2
(<i>d</i> × <i>k</i> / <i>d</i>) (k/ <i>d</i>)	13:1	45†	2	2	—
BALB/c × <i>A</i> → BALB/c	40:1	3	2	51†	1
(<i>d</i> × <i>k</i> / <i>d</i>) (<i>d</i>)	13:1	5	3	35†	2
Normal mice					
BALB/c	40:1	2	0	60†	2
(<i>d</i>)	13:1	—	1	22†	5
<i>A</i>	40:1	98†	2	—	—
(k/ <i>d</i>)	13:1	80†	1	—	—
C3H	40:1	97†	3	4	1
(<i>k</i>)					

*Recipient mice were irradiated with 925 rads and reconstituted with 2 × 10⁷ twice anti- θ plus C treated bone marrow cells. Chimaeras were infected 10 weeks later with about 10⁷ plaque forming units of vaccinia virus WR⁷. Their spleen cells were typed for *H-2*; for both chimaeras > 95% of the lymphocytes were lysed by anti-*K^d* and anti-*K^k* antiserum. Virus specific cytotoxic T cells were tested for 6 h on infected or uninfected target cells⁹. The spontaneous release from *L* cells was about 25%, from *D2.GD* macrophages about 45%.

†The results are given as means of triplicate determinations that were corrected for spontaneous release; water released about 80% of the incorporated ⁵¹Cr and this was taken as 100% release; s.e.m. were smaller than 5%.

‡Significantly different from relevant controls (*P* < 0.01).

Table 2 TNP-specific cytotoxic activity generated by $F_1 \rightarrow$ Parent or $F_1 \rightarrow F_1$ chimaeric lymphocytes *in vitro*

Responder* lymphocytes (H-2 type)	Stimulator lymphocytes	Target cells†	% Specific ^{51}Cr release from targets‡	
			TNP	Normal
Chimaeric mouse BALB/c $\times$ A (d $\times$ k d) (k d)	TNP-BALB/c $\times$ A (d $\times$ k d)	C3H (k) D2.GD (d b)	62 <1	<1 <1
Normal mouse BALB/c (d)	TNP-BALB/c (d)	BALB/c (d) D2.GD (d b)	93 28	47 18
Chimaeric mouse BALB/c $\times$ C3H $\times$ C57BL/6 BALB/c (d $\times$ b) (k $\times$ d)	TNP-C3H $\times$ C57BL/6 (k $\times$ b)	C3H (k) C57BL/6 (b)	65 <1	8 <1
Normal mice C3H (k) C57BL/6 (b)	TNP-C3H (k) TNP-C57BL/6 (b)	C3H (k) C57BL/6 (b)	61 90	<1 <1

*Chimaeras were prepared and typed for H-2 as in Table 1. Spleen cells (10^7 ml^{-1}) were cultured for 5 d with trinitrobenzene sulfonate (TNBS) coupled stimulator spleen cells ($5 \times 10^5 \text{ ml}^{-1}$) irradiated with 1,000 rad as described previously¹².

†Spleen cells were (10^6 ml^{-1}) cultured for 3 d in the presence of $10 \mu\text{g ml}^{-1}$ bacterial lipopolysaccharide, collected and coupled with TNBS and labelled with Cr^{51} (ref. 12). The attacker to target cell ratio was 50:1.

‡Values are means of duplicates corrected for spontaneous release. The 100% release was determined by freezing and thawing the cells 3 times. s.e.m. values were smaller than 5%. The 4-h time point is given.

TNP (Table 2). This indicates that stem cells differentiate in the irradiated recipient to acquire H-2 (C) restricted specificity that is host-dependent but independent of the H-2 type which the lymphocytes express themselves. The results from neonatally tolerant mice can be similarly explained⁹.

These results are compatible with the general idea of 'adaptive differentiation'¹⁰ and agree with similar data of Bevan¹¹, apparently implying that the H-2 type of the host in which T lymphocytes differentiate governs the specificity of subsequent H-2 restriction. One would therefore not expect parental strain cells depleted of alloreactivity to lyse antigen-modified fully allogeneic cells. Schmitt-Verhulst and Shearer¹³ showed that

lymphocytes of A origin which were deprived of anti B-alloreactivity by bromodeoxyuridine (BUdR) and light could not be sensitised specifically against B-TNP. However, their data and ours disagree with those of Wilson *et al.*¹⁴, who found that lymphocytes of A origin that were deprived of anti-B alloreactive cells by filtration through irradiated F_1 animals could be sensitised specifically against B-TNP. This discrepancy cannot yet be resolved, but the different reactivities may have arisen from incomplete tolerance of the filtered lymphocytes or differences in the method of TNP modification.

Our results indicate that the chimaeric host determines the H-2 restricted specificity of cytotoxic T cells; they illustrate clearly

Table 3 Virus specific cytotoxic T cell activity generated in adult thymectomised irradiated C57BL/6 mice that were reconstituted with anti- θ treated F_1 bone marrow cells and transplanted with irradiated F_1 thymus of BALB/c $\times$ C57BL/6 origin.

Bone marrow donor	Thymus donor	Irradiated recipient	Lymphocyte to target ratio	% Specific ^{51}Cr release from target cells			
				MC57G(b) Vaccinia treated	Normal	D2(d) Vaccinia treated	Normal
BALB/c $\times$ C57BL/6 (d $\times$ b)	BALB/c $\times$ C57BL/6 (d $\times$ b)	C57BL/6 (b)					
Thymus tissue detectable in kidney				35*	2	60†	5
				13:1	4	21†	3
				4:1	1	9	6
Thymus tissue not detectable in kidney				3	1	4	5
				13:1	2	2	2
Vaccinia virus infected control mice							
C57BL/6 (b)				40:1	95†	6	4
				13:1	45†	2	2
BALB/c (d)				40:1	5	100†	2
				13:1	2	55†	3

Recipient mice were thymectomised, 1 week later irradiated (950 rads) and reconstituted with twice anti- θ plus C treated bone marrow cells of F_1 origin: irradiated (900 rads) thymus lobes from 6–8-week-old F_1 donors were transplanted under the kidney capsule. These thymus chimaeras were infected about 3 months after reconstitution. 6 d later the animals were killed, the spleen cells typed for H-2 (> 95% were positive for H-2^b and H-2^d) and tested for cytotoxic activity in a 16-h test. Absence of the thymus of the host and presence or absence of thymic tissue under the kidney capsule were controlled histologically.

*Means of triplicate determinations; values are corrected for spontaneous release (MC57G: 26%, D2: 40%) and water release was taken as 100%.

†Significantly different from controls ($P < 0.05$).

that peripheral tolerance to H-2 is not sufficient to allow recognition by T cells of this same H-2 together with antigen. Thymus transplantation experiments indicate that it is the H-2 type of the thymus epithelial cells which determines the H-2 antigens treated as self by H-2 restricted T cells (Table 3). Adult thymectomised, irradiated C57BL/6 mice were reconstituted with bone marrow of BALB/c $\times$ C57BL/6 origin and transplanted with two irradiated thymus lobes of BALB/c $\times$ C57BL/6 origin under one kidney capsule. When infected with vaccinia virus 3 months later, cytotoxicity was generated for both parental H-2 type infected targets. This is in contrast with the results from $F_1 \rightarrow$ Parent irradiation bone marrow chimaeras, where activity was formed for the compatible recipient parental H-2 type only.

These two models differ with respect to the thymus of the two chimaeras and we may therefore conclude that the radioresistant part of the thymus, probably the H-2 specificity of the thymic epithelium, determines what differentiating T cells learn to recognise as self-H-2. Since thymectomy was histologically complete, and since virus-specific cytotoxic activity was generated for both infected H-2^b and H-2^d targets, and not for H-2^b alone, it follows that the transplanted thymus induced T cell maturation. The anti- θ plus C treatment was complete in depleting T cells, otherwise one would have expected that mature contaminating F_1 T cells would have given rise to a significant level of cytotoxicity in the second chimaera from the same group of animals, where no histological or functional thymus reconstitution was achieved. Therefore, it seems that precursor T cells learn to recognise as self the H-2 antigens expressed by the radioinsensitive portion of the donor thymus, that is, thymus epithelium. This capacity for self recognition differentiates independently from the H-2 haplotype of precursor T cells and independently from recognition of non-H-2 antigens.

Can these results best be explained by the single or dual recognition model? Lymphocytes from F_1 (A $\times$ B) $\rightarrow$ P(A) chimaeras generate antigen-specific killer T cells against H-2-compatible targets of parent A only, despite the fact that they were exposed to antigen in association with both A and B parental H-2 antigens. This could be explained by Jerne's speculation¹⁵ that T cells of a mouse A evolve from proliferating and mutating T cells that were specific for A to express specificity for 'different from A'. Within such a concept the one receptor hypothesis, which suggests that H-2 restriction results from an association of non-H-2 antigen at the level of induction could explain this experimental finding only if the rule were made that the specificity spectrum for modified self generated in thymus A cannot overlap with that generated in thymus B. This rule is unpredicted and unlikely, but cannot be excluded.

Because there is no precedent for the H-2 haplotype of the thymus restricting T cell-specificity (except perhaps for *Ir* gene effects), the explanation that T cells have independent receptor sites for thymus epithelial H-2 and for non-self antigen, and operate through dual recognition may be the more attractive one. Whether the two recognition structures are on one or two membrane molecules is not clear. These results and the concept that self-specificity is selected for in the thymus have important theoretical and practical implications for further experimentation. They may also provide an improved rationale for clinical attempts at reconstitution of immunodeficiency in humans. If the presented results are correct and apply to humans, it is predicted that (except for pure thymus hormone deficits) reconstitution of immunodeficient patients with completely HLA incompatible thymus epithelium or lymphohaemopoietic stem cells may result in differentiation of mature T cells that cannot interact with other T cells or B cells because they were taught in the thymus to recognise HLA structures as self that are not of their own HLA type. Therefore, functional immunological reconstitution of thymus and/or stem cell deficient patients with immunoincompetent thymus tissue or liver cells, for example, of foetal origin (to avoid graft versus host reactions), may depend on recipient and donor sharing at least one HLA-A or B and D haplotype.

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Cell surface changes in alloantigen activated T lymphocytes

MANY interactions which take place between T lymphocytes and other cells are presumably mediated at the level of the cell surface. In a mixed leukocyte culture (MLC), interaction between allogeneic lymphocytes results in transformation of the responding small T lymphocytes to large blast-like cells and their development into killer, helper¹ and suppressor lymphocytes². Some blasts subsequently revert to small memory or 'secondary-type' lymphocytes³. An important question is whether significant changes occur in T lymphocyte membrane proteins as a result of these interactions. We have attempted to answer this question by comparing iodine-labelled surface proteins of the following cells: T lymphocytes before stimulation, blast cells and small lymphocytes purified by Ig velocity sedimentation from an MLC on day five, and reversion T lymphocytes following an additional 5 d of culture of the separated blast T lymphocytes. The results demonstrate that iodine-labelled components change during blast transformation and provide evidence for membrane differences between 'secondary-type' T lymphocytes and unsensitised cells.

Figure 1 shows radioactivity profiles resulting from co-electrophoresis of NP-40 extracts of ¹²⁵I-labelled murine thymus and spleen and ¹³¹I-labelled nylon column purified spleen T cells on a 5% acrylamide, 0.1% sodium dodecyl sulphate (SDS) gel. When analysed on these 5% gels, both spleen and nylon column purified T cells, Fig. 1b show three distinct peaks of radiolabelled proteins which have molecular weights of approximately 200,000 (200K), 187K, and 170K. (Preliminary SDS gel studies of plasma membrane preparations (G. Sundharadas, to be published) from ¹²⁵I-labelled cells also show three peaks in the 170K to 200K molecular weight

range). The presence of these peaks in the thymus preparation (Fig. 1a) and in nylon wool purified cells and the absence of any of the three peaks in macrophage or nude mouse spleen cell preparations (data not shown), are consistent with these being specific T cell-associated peaks. Trowbridge *et al.*⁴, using a 7.5–15% gradient gel, have reported the presence of a T cell-specific protein of molecular weight 200K in preparations similar to ours. The peak of molecular weight 240K in Fig. 1b

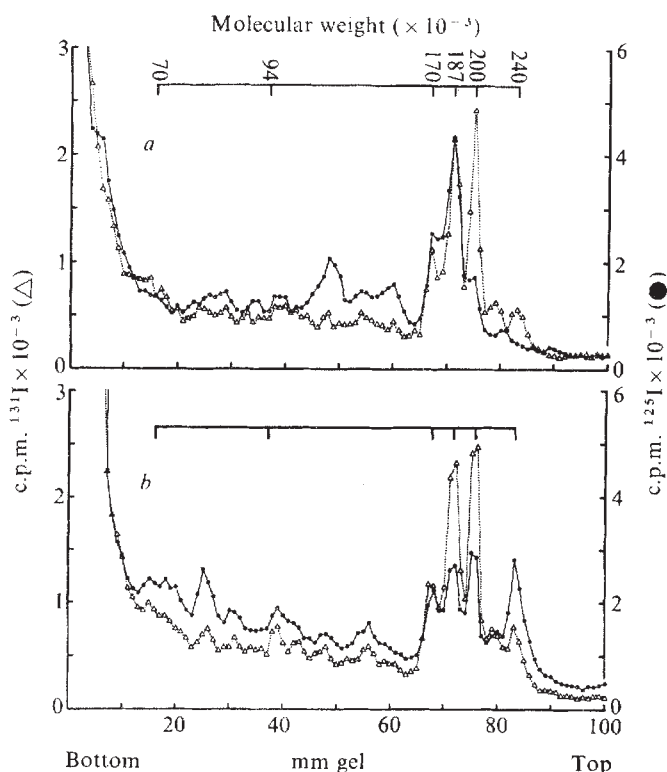


Fig. 1 NP-40 extracts of ^{125}I -labelled murine spleen and thymus and ^{131}I -labelled nylon column purified cells electrophoresed on SDS-polyacrylamide gels. Single cell suspensions were prepared by aseptically removing the spleen or thymus, and teasing them through a wire mesh into EHAA media. Aggregates were allowed to settle, the cells aspirated off and washed once in media. Erythrocytes were removed from the spleen cell suspensions by lysing with 0.15M NH_4Cl , 0.01M Tris Cl, pH 7.2. Nylon column purified T cells were prepared from spleen cell suspensions by passage over a nylon wool column according to Julius *et al.*⁷. Cells were washed three times in PBS, then $2\text{--}4 \times 10^7$ cells in 0.5 ml were iodinated with 1 mCi ^{125}I or ^{131}I (total concentration of iodide was $1 \times 10^{-6}\text{M}$) using the galactose-oxidase lactoperoxidase technique of Schenkein *et al.*⁸. Cell viability was determined before and after iodination; cell recovery was $>95\%$ and $>90\%$ of the cells were viable (by eosin staining) before and after iodination. After iodination, cells were washed three times with PBS, and lysed by addition of Nonidet P40 (Shell) to a final concentration of 0.2%. Nuclei were removed by centrifugation at 1,000g for 10 min. The supernatants were made 3% in SDS, 0.1M in β -mercaptoethanol, heated to 90°C for 3 min, and indicated samples co-electrophoresed on 6 mm $\times$ 110 mm, 5% acrylamide 13% bi-sacrylamide gels containing 0.1% SDS, according to Laemmli⁹. Electrophoresis was stopped when the tracking dye reached the lower edge of the gel, and the gels separated into 1 mm fractions using a Gilson gel fractionator model B-200 GMA-GCB. Scintillator fluid (3a70B, Research Products International, Incorp.) 3 ml, was added to each fraction and the fractions counted in a Packard tricar liquid scintillation spectrometer model 3330 using window settings of 50–1,000 (^{125}I), 200–1,000 (^{131}I) and a gain of 50% (^{125}I) or 6% (^{131}I). The net counts of ^{125}I were corrected for overlap of ^{131}I counts into the ^{125}I -channel. Approximate molecular weights of the peaks were calculated from their position relative to those of known standards. *a*, Extracts of ^{125}I -labelled thymus cells ($\bullet$) and ^{131}I -nylon wool cell extracts (Δ). *b*, Extracts of ^{125}I -labelled spleen cells ($\bullet$) and ^{131}I -nylon wool cell extracts (Δ).

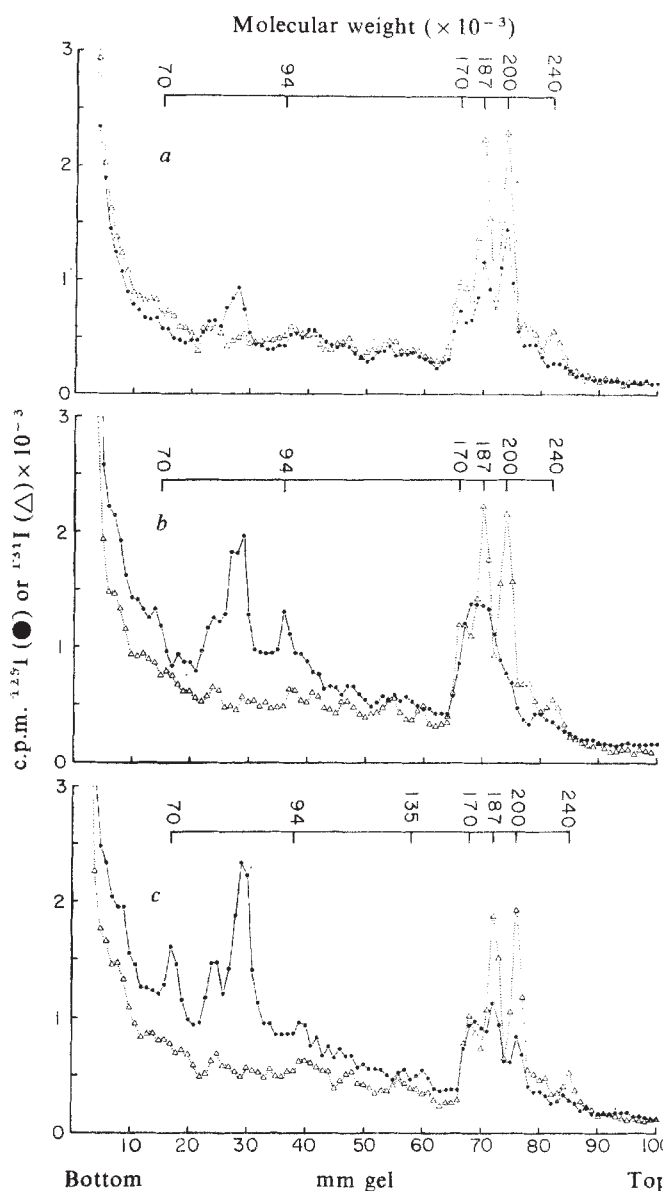


Fig. 2 NP-40 extracts of ^{125}I -labelled small lymphocytes, blasts, reverted cells and ^{131}I -nylon wool purified T cells, electrophoresed on SDS-polyacrylamide gels. Mixed leukocyte cultures were set up in 250 ml Falcon tissue culture flasks (50–75 ml media per flask), using culture conditions according to Peck and Bach¹⁰. Nylon wool purified T cells from B10.M mice were prepared as described in Fig. 1, and stimulated with allogeneic (B10.G) γ -irradiated (2,000R) spleen cells, using 2.5×10^6 responding and stimulating cells per ml. After 5 d in culture, cells were harvested, washed with PBS and separated into large, rapidly sedimenting 'blast' cells and small, slowly sedimenting lymphocytes on a 5–30% horse serum gradient by unit gravity velocity sedimentation¹¹. Cells were allowed to settle for 4 h, the gradient fractions checked microscopically, and fractions containing either pure blasts or pure small lymphocytes were pooled. Samples of each were washed three times in PBS, labelled with ^{125}I and extracted with Nonidet P-40, as described in Fig. 1. The remaining blasts were then cultured for 5 d in 50 ml Falcon tissue culture flasks, containing 10^7 cells in 10 ml EHAA medium, at 37°C in a 5% CO_2 atm, after which they were collected, washed three times, in PBS labelled with ^{125}I and extracts prepared as in Fig. 1. No blasts were evident in these 'reverted' cell cultures. The ^{125}I -labelled extracts were co-electrophoresed with ^{131}I -labelled extracts from nylon column purified T cells, as described in Fig. 1. *a*, Extracts of ^{125}I -labelled small lymphocytes from 1g gradient ($\bullet$) and ^{131}I -nylon wool cell extracts (Δ). *b*, Extracts of ^{125}I -labelled blast cells from 1g gradient ($\bullet$) and ^{131}I -nylon wool cell extracts (Δ). *c*, Extracts of ^{125}I -labelled reverted cells ($\bullet$) and ^{131}I -nylon wool cell extracts (Δ).

which is present in the spleen cell preparation is probably a B cell-associated protein, as it is greatly reduced in extracts made from cells passed through nylon wool columns (Fig. 1b), and is absent in macrophage and blast cell extracts.

Figure 2a and b shows the profiles of nylon separated lymphocytes compared with extracts of labelled small lymphocytes and blast cells, respectively, after separation on a 1g gradient from a 5-d-old MLC. Blast cells activated by allogeneic cells (Fig. 2b) have the three distinct peaks of molecular weights 200K, 187K and 170K, replaced by a broad band of radiolabelled proteins covering the molecular weight range of 170–200K. In addition, a new peak or greatly increased component of 94K has appeared and several changes in the intensity of other peaks have occurred. The small lymphocytes from the 1g gradient retain the three distinct peaks (200, 187 and 170K) present in the starting nylon column purified cells, as well as most of the other starting cell peaks.

In contrast to the blast cells, reverted cells (Fig. 2c) have three discernible peaks of radioactivity in the 170–200K molecular weight range. The 200K and 187K peaks are the same as the peaks in the nylon column purified cells, but the third peak (170K in the nylon column cells) is reproducibly broader and shifted to slightly higher molecular weight. There is an increase in the amount of the 200K peak in reverted cells compared to blast cells. In addition, the 94K peak in the blast cells is greatly reduced or missing from the reverted cells, while a peak of 70K is prominent in the reverted cells but not in the blast cells. In comparison to the starting nylon wool cells, the reverted cells have different proportions of the 170K, 187K and 200K peaks, and peaks of 70K and 90K have appeared which are absent or present in low amounts in the starting cells.

The experiments presented demonstrate that T cells obtained from the blast cell fraction of a 1g gradient that have undergone functional change after allogeneic stimulation have different cell surface 125 I-labelling patterns from those of a starting population of T cells obtained from nylon wool or small lymphocytes obtained from the 1g gradient. The differences are consistent with the surface differentiative changes which take place as a small lymphocyte develops into a blast cell. These changes, however, may be associated with the cell cycle or represent alterations in the accessibility to iodination of common components due to changes in other components. They may also reflect expression of new blast-specific surface structures or modification of molecules existing in the non-stimulated cell, for example, by glycosylation. Also, selection of a clonal type, where cells of different responding clones give rise to functionally similar blasts which have different surface patterns, can be ruled out because we have observed the same changes where stimulating cells from different strains were used (unpublished data). Further, one might not expect variation in the molecular weight (as measured in SDS) of a given membrane product in different clones of cells with the same function.

More difficult to evaluate is whether these changes could reflect selection, during culture, of one or more functionally distinct T cell subpopulations with the observed 125 I-banding patterns. We regard this as unlikely in view of our current understanding that the T cell subpopulations present and responding in the initial T cell population^{5,6} are the same as and occur in similar proportion (A. Senik & B. R. Bloom, personal communication) to those found in the blast fraction. The interpretation of our results as a selection process could be clarified if the starting population could be more specifically defined, by raising antisera to the proteins that differentiate the starting small lymphocytes, the blasts and reversion lymphocytes and using these sera to dissect the various populations.

The blast T lymphocyte population analysed on these gels consists of several functionally different T cell populations. The majority of T lymphocytes present in the blast cell fraction are probably proliferating helper T cells, while a small percentage are cytotoxic T lymphocytes, and some suppressor T lymphocytes may be present. We are undertaking further experiments to determine if these functionally different blast

cell populations also differ in their cell surface components.

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Cyclophosphamide inhibited B cell receptor regeneration as a basis for drug-induced tolerance

A WIDE range of immunosuppressive agents can facilitate the induction of specific tolerance by thymus-dependent antigens. In the case of cyclophosphamide (CY), this involves T cells (including the generation of suppressor T cells), whereas B cells remain relatively unaffected^{1–3}. On the other hand, CY lowers the B cell tolerance threshold to thymus-independent (TI) antigens^{4,5}, a characteristic not shared by other alkylating agents (melphalan and chlorambucil) or immunosuppressive drugs (6-mercaptopurine, azathioprine and A-methopterin) (J. G. Howard and C. Hale, in preparation). Although high doses of CY induce selective depletion of B cells^{6,7}, its immunosuppressive potential *in vivo* or *in vitro* is manifest with doses well below this cytotoxic threshold (F. L. Shand, in preparation), which is not the case with melphalan and chlorambucil. Consequently, the B cell tolerance-promoting and reversible immunosuppressive activities of CY might be dissociable from its cytotoxicity. We have investigated the ability of splenic B lymphocytes from CY-injected mice to cap and subsequently regenerate their surface immunoglobulin (SIg) receptors following treatment with anti-immunoglobulin. Re-expression of B cell SIg was grossly impaired following treatment with CY, but not with other immunosuppressive agents. This finding provides a possible explanation for the increased susceptibility of B cells to tolerance induction with TI antigens.

Mice of both sexes from various strains (BALB/c, CBA/Lac, CBA/H and CBAXC57BL)F₁ were used between 3 and 6 months of age. Cyclophosphamide monohydrate (Koch-Light), melphalan, chlorambucil and 6-mercaptopurine (Wellcome) were injected intraperitoneally at 150, 10, 30 and 75 mg per kg, respectively. Melphalan and chlorambucil were used at their maximally tolerated doses; in the case of CY, this exceeds 300 mg per kg. Spleen cell suspensions were treated with rhodamine-labelled rabbit-anti-mouse Ig (supplied by Dr L. Hudson) for 45 min at 37 °C. After three washes, 1×10^7 cells were cultured in Marbrook chambers and samples collected periodically for indirect immunofluorescent staining in 0.13% sodium azide using the (Fab')₂ fragment of rabbit-anti-mouse Ig (pre-

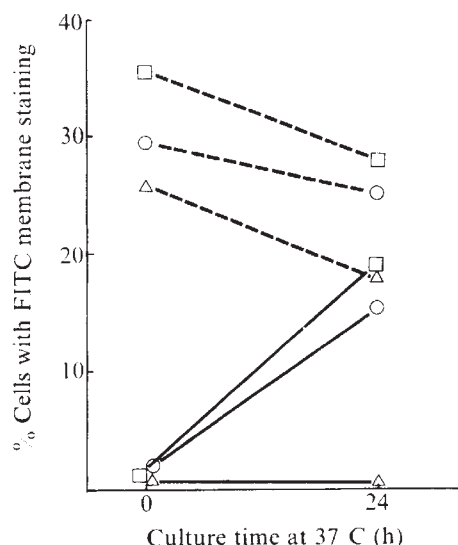


Fig. 1 Comparison of SIg receptor regeneration of spleen cells from normal ($\square$) melphalan-injected (10 mg per kg) ($\circ$) or CY-injected (150 mg per kg) ($\triangle$) mice, after treatment with anti-Ig serum. Spleen cells (2.5×10^7) were incubated in 0.5 ml of rhodamine-labelled rabbit-anti-mouse Ig serum (1/40) for 45 min at 37 °C. Suspensions were washed three times and 1×10^7 cells cultured in RPMI + 5% foetal calf serum in Marbrook chambers for 24 h at 37 °C in 5% CO_2 . Cells were collected and stained in 0.13% sodium azide with an indirect immunofluorescence assay using a (Fab')₂ fragment of rabbit-anti-mouse Ig (1/20) followed by a FITC-labelled sheep-anti-rabbit Ig (1/20). The percentage of total lymphocytes expressing new FITC labelled SIg was based on counts of 200–300 cells. Dashed lines represent the levels of SIg-bearing cells in control preparations which were not treated with rhodamine-labelled anti-Ig serum.

pared by pepsin digestion and fractionation on ultragel Ac-44) followed by fluorescein-labelled sheep-anti-rabbit Ig (Wellcome Reagents Ltd). This protocol enabled us to monitor both the frequency of capping and the reappearance of new SIg. The use of a (Fab')₂ fragment excluded the possibility of detecting Fc receptors. In enumerating those B cells which expressed new SIg, we recorded as positive only those cells which showed typical ring membrane fluorescence with fluorescein in the absence of rhodamine.

Whereas normal spleen suspensions after anti-Ig treatment had levels of B cells expressing new SIg which were 40 and 75% of control values at 6 and 24 h respectively, a corresponding increase in B cells expressing new SIg was not detected in spleen suspensions from mice injected with

CY 20 h earlier (Table 1). This result was obtained consistently in four experiments. Although a 30% reduction in the number of B cells recovered from CY-injected mice was always found, the frequencies of cells capping were essentially similar in either type of suspension. The percentage viability of cells from CY-injected mice was only marginally reduced after 24 h of culture as compared with normal cells. Analogous impairment of SIg receptor regeneration has also been reproduced with normal spleen cells previously treated with CY activated by liver microsomes *in vitro* (F. L. Shard, in preparation).

In other experiments, spleen cells from CY-treated mice were compared with those from normal or melphalan-treated mice. Figure 1 shows typical impairment of SIg regeneration in CY- but not melphalan-injected mice. In another experiment, neither chlorambucil nor 6-mercaptopurine caused any impairment of SIg receptor regeneration. Thus, the failure of B cells from CY-injected mice to regenerate their SIg receptors is a distinctive sequel to treatment with this drug not shared by the other immunosuppressive agents.

To ascertain whether this abnormal behaviour of B cells 20 h after CY injection was a reversible phenomenon, spleen cells from mice injected with CY 1, 3 or 7 d previously were examined for their ability to regenerate SIg after anti-Ig treatment. Partial recovery of SIg regeneration was found 3 d after CY and full recovery by 7 d (Fig. 2a). The immune status of parallel groups of mice was established by challenge with either levan or sheep red blood cells at the same time intervals after CY treatment. The levels of SIg recovery correlated with the extent of immune response following challenge with both T-dependent and T-independent antigens at the same times (Fig. 2b).

In a final variant of these experiments, the incubation

Fig. 2 a, Comparison of SIg receptor regeneration of spleen cells obtained from mice injected 1, 3 or 7 d previously with 150 mg per kg CY after treatment with anti-Ig serum. Percentage of cells showing typical membrane ring fluorescence; before anti-Ig treatment (open columns), after anti-Ig treatment and 24 h of culture (shaded columns) and without anti-Ig treatment after 24 h of culture (stippled columns). Other details as in Fig. 1. **b**, Plaque-forming cell (PFC) response to sheep red blood cells (SRBC) (open columns) and levan (shaded columns) in groups of mice injected 1, 3 or 7 d previously with 150 mg per kg CY ($n = 5$). Mice were immunised with 5×10^8 SRBC or 10 μg levan i.v. and direct PFC assay performed 5 d later. SRBC were conjugated with levan as described by Miranda¹³.

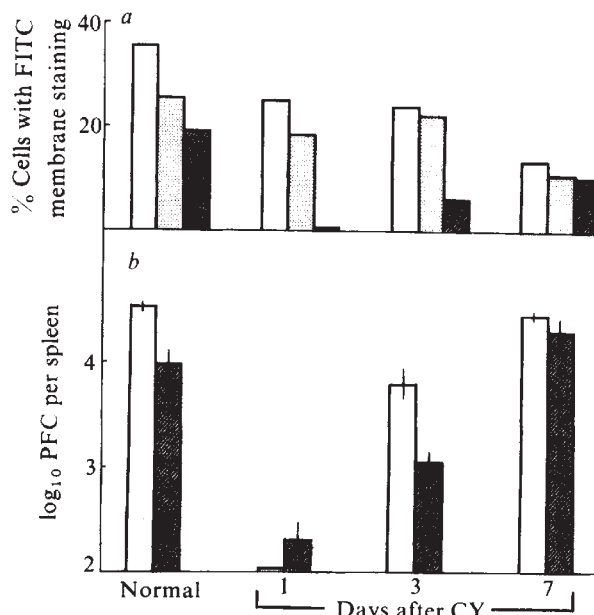


Table 1 Percentage of total lymphocytes expressing new SIg at various times after anti-Ig treatment

% Cells showing membrane staining with FITC-anti-Ig only at (h)	Normal mice		Mice injected with 150 mg per kg CY 20 h previously	
	RAM-Rh*	Medium only	RAM-Rh	Medium only
0	<1.0	37.9	<1.0	22.8
6	14.6	35.1	2.8	23.3
24	25.2	33.3	1.0	16.6
% caps immediately after	91	—	93	—
RAM-Rh treatment				
% viability at 24 h†	55	58	45	49

*RAM-Rh, spleen cells were incubated in rhodamine-labelled rabbit-anti-mouse Ig for 45 min at 37 °C and washed three times.

†Determined by Trypan blue dye exclusion. Other details as in Fig. 1.

Table 2 The inability of CY-treated spleen B cells to regenerate their SIg is irreversible

% Cells showing membrane staining with FITC-anti-Ig only at (h)	Spleen cells from			
	Normal mice	Mice injected with 150 mg/kg CY 24 h previously	RAM-Rh*	Medium only
0	<1.0	40.1	<1.0	31.2
24	24.6(79)	38.5(85)	1.7(63)	29.5(73)
48	18.9(53)	32.7(65)	1.2(44)	18.1(49)
72	16.3(27)	21.8(30)	1.0(25)	13.5(26)

Figures in parentheses, % viability, estimated by Trypan blue dye exclusion.

*RAM-Rh, spleen cells were incubated in rhodamine-labelled rabbit-anti-mouse Ig for 45 min at 37 °C and washed three times.

Other details as in Fig. 1.

period after anti-Ig treatment was increased to 72 h, to determine whether or not the impairment of SIg regeneration was durable. No receptor regeneration of CY-treated B cells was observed even after 72 h of culture, despite cell viabilities which were comparable to those of control groups (Table 2).

It has been suggested that CY-induced tolerance involves death of lymphocytes triggered by antigen either because of a block in their normal differentiation sequence or a selective vulnerability of the S or G₂ phases of the cell cycle⁸. This view is weakened by the finding that CY promotion of tolerance with T-independent antigens depends on their tolerogenicity rather than immunogenicity (ref. 5 and J. G. Howard and C. Hale, in preparation). An attractive and likely alternative is that the B cell tolerance-promoting effect of CY is due to an impairment of SIg receptor regeneration following interaction with multivalent antigens, assuming that the sequelae are similar to those following anti-Ig interaction. The fact that other immunosuppressive drugs seem incapable of reproducing this effect and of promoting tolerance strongly supports such a view. Moreover, the finding that B cells from CY-injected mice containing endocytosed rhodamine-labelled anti-Ig were still abundantly present after 24 h of culture further substantiates a lack of cytotoxic deletion.

Anti-Ig treatment of immature B cells in foetal liver, neonatal spleen and adult bone marrow cells^{10,11} causes an irreversible disappearance of SIg which is not found with mature B cells. This may explain the ease with which immature B cells are tolerised by multivalent antigens¹⁰. A similar result has been obtained by the prolonged exposure of B cells to certain polymeric antigens^{11,12}. We suggest that CY induces a temporary reversion to a state of functional immaturity analogous to that of B cells in foetal or newborn mice, during which their interaction with antigen leads to tolerance alone. We are currently seeking further evidence of indissociability between impaired SIg receptor regeneration and reversible immunosuppression of B cells induced by CY, as well as the nature of the underlying metabolic events.

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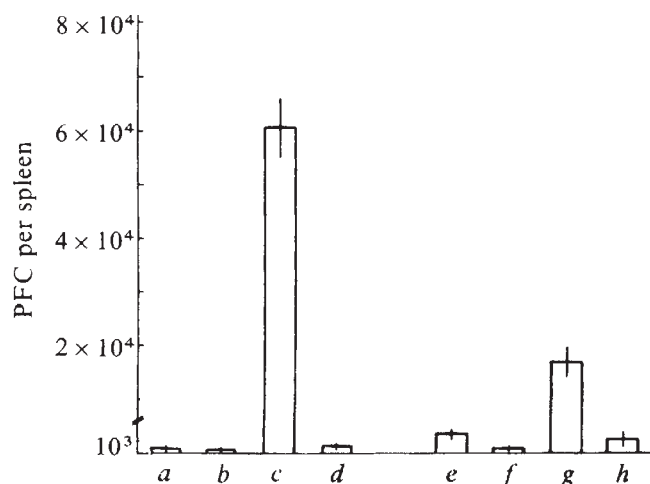
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Specific immune response enhancing factor in serum of immunised mice

ONE of the central problems of immunology is the mechanism by which regulatory thymus derived T cells—T helper, T amplifier and T suppressor cells—interact with each other and with the precursor cells of the antibody-forming B lymphocytes. Various mediators produced by T cells acting directly or indirectly on B cells have been described. Some of these factors have been shown to enhance^{1–4} or to suppress^{5,6} the function of B cells either in an antigen-specific^{1,2,5} or in a nonspecific^{3,4,6} manner. Immune response-enhancing factors produced *in vitro* have been shown to replace T helper or T amplifier cell function in thymus-dependent antibody formation *in vivo*^{1,2} and/or *in vitro*^{1–4}. We show here that the serum (S₄) of mice injected 4 h previously with a super-optimal dose of sheep red blood cells (SRBC) contains an antigen-specific principle—specific immune response enhancing factor (SIREF)—which enhances the humoral immune response up to 60-fold when injected before a sub-optimal antigen dose into mice but not in mice injected with an optimal dose of antigen (2 × 10⁸ SRBC). SIREF could not be produced in athymic nude mice and SIREF produced in BALB/c (H₂d) or C57Bl (H₂b) mice acted in either strain, but not in athymic mice (Table 1). These results indicate that SIREF(1) may be released from T cells (or from B cells which require T-cell cooperation), (2) acts through the H₂ barrier, and (3) does not act directly on B cells.

As shown in Fig. 1, SIREF is antigen specific—if produced

Fig. 1 Antigen specificity of SIREF. BALB/c mice were injected i.v. with 10⁸ SRBC or 10⁸ HRBC (horse red blood cells). Serum collected 4 h later (S₄ SRBC or S₄ HRBC respectively) was injected i.v. (0.1 ml per mouse) in mice immunised with either SRBC (10⁵ SRBC per mouse i.v.) or HRBC (5 × 10⁴ HRBC per mouse i.v.). S₄ was given 2 h before the antigens. Controls were injected with either S₄ SRBC, S₄ HRBC, 10⁵ SRBC or 5 × 10⁴ HRBC. The IgM PFC in the spleen of the mice were detected 6 d later. Each value represents the mean of PFC per spleen detected in 6 animals on day 6. The vertical bars represent the standard deviation of the mean. HRBC-immunised mice were tested for anti-HRBC-PFC, SRBC immunised mice were tested for anti-SRBC-PFC. a, S₄ SRBC (10⁵); b, S₄ SRBC; c, SRBC (10⁵) + S₄ SRBC; d, SRBC (10⁵) + S₄ HRBC; e, S₄ HRBC; f, HRBC (5 × 10⁴) + S₄ SRBC; g, HRBC (5 × 10⁴) + S₄ HRBC; h, HRBC (5 × 10⁴) + S₄ SRBC.



in SRBC-injected mice SIREF enhances the immune response in SRBC immunised recipients but not in mice immunised with horse red blood cells (HRBC) and vice versa. The enhancing effect of S_4 HRBC was, however, significantly less marked than that found in animals receiving S_4 SRBC and SRBC. Rabbit anti-SRBC antibody bound covalently to Sepharose removed up to 90% of SIREF activity from S_4 SRBC (T.D. and H.N., in preparation). This finding again shows the specificity of SIREF and suggests that SIREF has to contain such antigenic determinants.

SIREF is not simply an antigen or an immunogenic degradation product of SRBC. This view is supported by the following findings: (1) 0.4 ml of S_4 derived from either strain of mice was even less effective than 0.1 ml of S_4 in enhancing the plaque-forming cell (PFC) response, (2) when injected alone 0.4 ml S_4 was no more immunogenic (280 ± 93 PFC) than 0.1 ml of S_4 (314 ± 119) as tested 6 d later and (3), serum derived from antigen-injected nude mice did not show significant SIREF activity.

SIREF does not alter the initiation of the immune response but enhances and prolongs the antibody formation (Fig. 2). The effect of the factor in the primary immune response is restricted to the IgM response. Preliminary experiments (data not shown) indicate that SIREF counteracts a mechanism which determines both the magnitude and persistence of the IgM response. A similar activity has been described by Henry and Jerne⁷, who obtained identical results injecting an active principle isolated from the serum of SRBC-immunised mice 4 d after immunisation. They claimed that the serum activity was due to IgM anti-SRBC antibodies present in their preparation. In our experiments, serum containing SIREF obtained 4 h after immunisation was completely free of anti-SRBC antibodies (as tested by anti-SRBC antibody titration) and SIREF could be detected (in a much smaller amount) up to 4 d after immunisation. We suggest that the 'regulation of the immune response by IgM antibodies' as proposed by Henry and Jerne should in fact, to accommodate our findings, be considered as 'regulation of the IgM response by SIREF'. This suggestion is further supported by the findings (1) that SIREF is not dialysable, (2) that in ammonium sulphate treatment of serum the

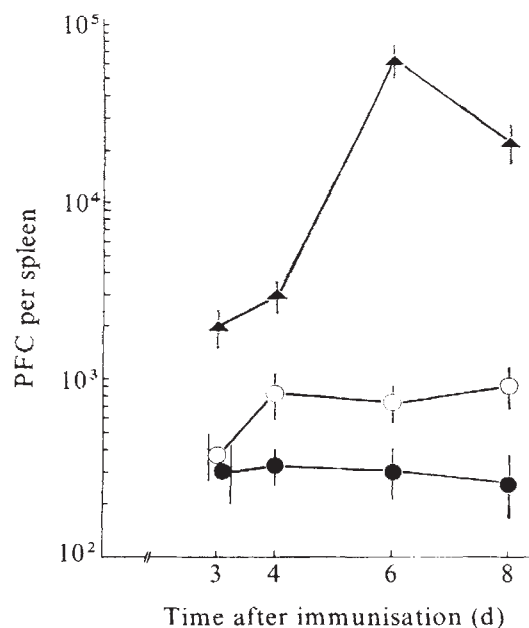


Fig. 2 Effect of SIREF on the kinetics of the IgM response. BALB/c mice 6–8 weeks old were injected with 10^6 SRBC; 0.1 ml serum collected 4 h later (S_4) was injected i.v., 2 h before immunisation of syngeneic animals with 10^6 SRBC i.v. Controls were injected with either S_4 or 10^6 SRBC i.v. At the times indicated direct IgM and indirect IgG PFC were assayed by the Jerne technique in the modification of Cunningham and Szenberg¹⁰ and as described in detail previously¹¹. Each point represents the mean of direct PFC detected in the spleens of 6 animals $\pm$ standard deviation. IgG PFC could not be detected in either group of mice. Anti-SRBC hemolysins titers paralleled the PFC response presented here (Data not given here). $\blacktriangle$, S_4 + SRBC; $\circ$, SRBC; $\bullet$, S_4 .

SIREF coprecipitates with the globulin fraction, and (3), SIREF was enriched in the conditions used by Henry and Jerne⁷ (H.N. and T.D., in preparation).

There are some similarities between SIREF and a serum factor described by Paraskevas *et al.*^{8,9}, who suggested that their factor is an antigen–IgM antibody complex released from B cells, cytophilic for a subset of T cells. This assumption was supported by the finding that spleen T cells derived from mice injected with SRBC 6 h previously and subsequently transferred into irradiated syngeneic animals enhanced the T cell-dependent IgG response (up to sevenfold) and the IgM response (up to two- to threefold) to SRBC. Paraskevas *et al.* assumed that at the time of the transfer the T cells were already armed with antigen–antibody complexes. SIREF, in contrast, does not affect the primary IgG response to either dose of SRBC but enhances dramatically (up to 60-fold) the IgM response when injected in normal animals immunised with a suboptimal antigen dose. Injection of SIREF does not induce immunological memory but injection of SIREF with a suboptimal dose of antigen induces development of memory cells of IgG type in a much higher amount than injection of the animal with the antigen alone (data not shown).

Several questions remain to be clarified: (1) is SIREF identical with Henry and Jerne's factor believed at that time to be IgM anti-SRBC antibody? (2) Is SIREF identical with the serum factor of Paraskevas *et al.*, and the discrepancies detailed above due only to differences in the ability to produce and/or to test the factor activity? (3) Are the three factors essentially identical, but according to our data released from T cells (T cell–receptor antigen complex) rather than from B cells (antigen–antibody complex)? The similarities in the production of SIREF, the factor obtained by Henry and Jerne or by Paraskevas and Lee and the finding that SIREF could not be produced in mice lacking functioning T cells (nude mice) seem to support the latter possibility. However, the contribution of

Table 1 T-cell dependence of production and action and the lack of H_2 -restriction of SIREF

S_4 donor	S_4 recipient	PFC response per spleen
BALB/c	BALB/c	$64,560 \pm 7,544$
BALB/c	C57Bl	$47,200 \pm 9,512$
BALB/c	nu/nu	257 ± 109
C57Bl	C57Bl	$36,480 \pm 7,651$
C57Bl	BALB/c	$73,640 \pm 11,459$
C57Bl	nu/nu	203 ± 77
nu/nu	nu/nu	263 ± 141
nu/nu	BALB/c	$1,329 \pm 273$
nu/nu	C57Bl	$1,174 \pm 309$
Recipient of 10^6 SRBC	S_4 donor and recipient	PFC response per spleen
BALB/c	—	841 ± 176
C57Bl	—	689 ± 251
nu/nu	—	224 ± 64
—	BALB/c (0.1 ml)*	314 ± 119
—	BALB/c (0.4 ml)	280 ± 93
—	C57Bl (0.1 ml)	347 ± 88
—	C57Bl (0.4 ml)	360 ± 65
—	nu/nu (0.1 ml)	293 ± 141
—	nu/nu (0.4 ml)	224 ± 64

BALB/c, C57Bl or athymic nude mice were injected with 10^6 SRBC i.v. Serum collected 4 h later (S_4) was tested for SIREF activity and for its immunogenicity on injection i.v. either S_4 2 h before immunisation of the mice with 10^6 SRBC i.v. or S_4 alone. S_4 derived from either donor strain was tested in either recipient strain. The values represent IgM PFC detected on day 6 in the spleen of 6 mice per group $\pm$ s.d.

*Volume of S_4 injected shown in parentheses.

accessory cells in production and/or action of SIREF cannot be excluded.

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Transfer of the marker for morphologically transformed phenotype by isolated metaphase chromosomes in hamster cells

In spite of extensive investigations of the process of carcinogenesis *in vitro*, the basic mechanisms of the primary events are poorly understood¹. In general, it is found that the first manifestation of the transformed phenotype involves morphological alteration of the cells. This is usually followed by an ill-defined process resulting in several further changes in phenotype including the ability of the cells

metaphase chromosome⁴⁻⁹. Most of the initial work was done with the genes for hypoxanthine phosphoribosyl transferase and thymidine kinase, but dominant markers such as resistance to methotrexate and ouabain can also be so transferred⁹, as can the wild-type alleles for three auxotrophic markers¹⁰. In seeking to delineate the genetic elements of carcinogenesis, we have examined the ability of purified metaphase chromosomes from Chinese hamster ovary (CHO) cells to transfer the first recognisable phenotype observed during cell carcinogenesis, that is, morphological transformation. Because of the low frequency of chromosome transfer, an appropriate method for selection of cells (transferents) which have incorporated the relevant markers from the background of recipient cells is necessary. Our experiments were based on the assumption that if transformation gene(s) could be transferred to senescent cells, then they should be able to produce colonies, whereas the majority of the cells which do not receive the appropriate marker, will not form colonies. The results presented here show that senescent hamster cells can be rescued and become transformed by transfer of purified metaphase chromosomes from CHO cells; the frequency of this event is similar to that of single gene markers.

Primary Chinese hamster lung cells were subcultured through five generations, and cloned. When the cloned cultures had reached confluence (~15 further generations), they were split into two subcultures, in 100-mm plates, and grown again. At the end of this time the cells were growing slowly, and were obviously in senescence. As might be expected, each clone gave rise to different numbers of cells at this stage. Nevertheless, in this way, a number of sets of two plates, each pair containing similar numbers of cells at the same level of senescence, were available as recipients. Purified metaphase chromosomes were isolated from CHO cells and aliquots added to one plate from each clone, as previously described^{2,9}. The plates were incubated at 37 °C in α -medium¹¹ containing 15% FCS for 2 d. Cells were then trypsinised, counted, replated in the same

Table 1 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with purified metaphase chromosomes of CHO cells

Donor for chromosomes*	No. of cells plated $\times 10^5$ CHL clone no.†						No. of colonies obtained CHL clone no.‡						No. of colonies per no. of cells or cell equivalent chromosomes $\times 10^6$
	1	2	3	4	5	6	1	2	3	4	5	6	
1 CHO	3	5	10	1	2	4	2	6	13	1	0	5	10.4
2	4	4	10	2	3	5	0	0	0	0	0	0	<0.4
3 CHO							0	0	0	0	0	0	<0.1

*A total of 2×10^6 cell equivalent chromosomes was added per plate.

†At day 2 after treatment cells treated with chromosomes (line 1) or untreated cells (line 2) were trypsinised and counted. The cells were plated in 20 ml of α -medium containing 15% FCS, at a concentration of up to 5×10^5 cells per 100-mm plate. Incubation of chromosomes alone (line 3) was continued in the above medium.

‡Colonies obtained after incubation at 37 °C for 3 weeks.

to grow in agar (aga⁺), and to produce tumours in animals. In part because of the variety of phenotypes observed, but also because of the lack of suitable methods of analysis, it has been difficult to examine the process of transformation at the genetic level. We have recently initiated experiments designed to use chromosome transfer techniques to attempt such a genetic analysis^{2,3} and work in several laboratories has shown that single gene markers can be transferred from cell to cell at frequencies of 10^{-6} – 10^{-7} by means of the

medium and incubated at 37 °C for 3 weeks. Colonies were then counted and isolated using a stainless steel cylinder and trypsinisation. The results of the first such experiment are presented in Table 1 which shows that colonies were obtained in five of the six cultures treated with metaphase chromosomes (line 1), whereas no colonies were obtained when the senescent cells were plated without previous addition of metaphase chromosomes (line 2). No colonies were obtained when metaphase chromosomes were

Table 2 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with purified metaphase chromosomes of CHO cells

Donor for chromosomes*	No. of cells plated $\times 10^5$ CHL clone no.†					No. of colonies obtained CHL clone no.‡					No. of colonies per no. of cells or cell equivalent chromosomes $\times 10^6$
	11	12	13	14	15	11	12	13	14	15	
1 CHO	6	4	10	10	1	3	2	10	7	0	7.1
2 CHLP6	6	8	8	9	2	0	0	0	0	0	<0.4
3 CHOP6	7	5	8	8	2	0	0	0	0	0	<0.4
4	5	4	9	10	2	0	0	0	0	0	<0.4
5 CHO						0	0	0	0	0	<0.1

*, †, and ‡ as in legend to Table 1.

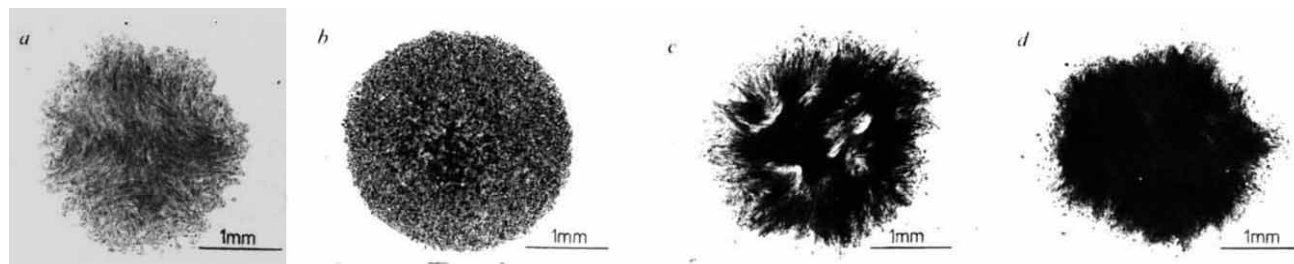


Fig. 1 Normal and transformed colonies. Normal recipient Chinese hamster lung cells (a), donor CHO cells (b) and two phenotypically different morphologically transformed Chinese hamster lung cells with metaphase chromosomes of CHO cells (c, d).

added to the plates in the absence of recipient cells (line 3). The results of a similar experiment, with two additional controls, are shown in Table 2. Again, colonies were obtained when CHO chromosomes were added to the senescent cells (line 1) and no colonies were found in the absence of chromosomes (line 4) and in the absence of recipient cells (line 5). We also prepared metaphase chromosomes from two primary strains developed from Chinese hamster lung and Chinese hamster ovary, both in their sixth transfer passage (1:4 splits), CHLP6 and CHOP6, respectively. As may be seen in Table 2 (lines 2 and 3), these chromosomes were also unable to rescue senescent cells.

The experiments described in Tables 1 and 2 provide strong evidence that CHO metaphase chromosomes can rescue senescent cells. Figure 1 shows the morphology of clones from two of these transferent clones, as well as that of the donor and primary recipient cells. It is clear that the rescued cells have been altered morphologically and have the appearance of transformed cells. This transformed phenotype is maintained on continued culture of these clones. Four of the transferents were karyotyped in detail with trypsin-Giemsa banding. Of 26 cells examined, 60% had a normal diploid karyotype, 15% showed loss of one or more chromosomes, and 25% showed structural rearrangements.

spontaneous transformation in the recipients, and also tend to rule out any mechanism of 'induction' of transformation in recipients due to addition of chromosomal material.

The frequency of appearance of transferents in all of the experiments (Tables 1-3) is similar to that observed for a variety of single gene markers^{2,9,10}. This fact, in addition to the localisation of the activity to one size class argues for the view that the transformation of recipient cells by metaphase chromosomes is caused by the transfer of a single gene.

Although the transferent cells obtained in these experiments show a transformed morphology, we have found that they do not form colonies in agar (aga⁻) or tumours when injected into recipient animals², but they can be converted into cells which do form colonies in agar (aga⁺) and which can produce tumours in animals, by a second addition of CHO metaphase chromosomes, and appropriate selection². It has not been possible to produce aga⁺ cells directly by addition of CHO chromosomes to primary cells. In terms of their ability to be converted to aga⁺, the primary transferents behave identically to cells which have been transformed by benzo(a)pyrene, or cells which have transformed spontaneously in culture². These results provide further confirmation that the rescued senescent cells are transformed, and that a gene involved in a first step in

Table 3 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with fractionated metaphase chromosomes of CHO cells

Chromosomal fraction*	No. of cells plated $\times 10^5$ CHL clone no.†				No. of colonies obtained CHL clone no.‡				No. of colonies per no. of cells or cell equivalent chromosomes $\times 10^6$
	21	22	23	24	21	22	23	24	
1 Large(A)	7	5	10	6	8	7	12	8	12.5
2 Medium(B)	4	6	9	7	0	0	2	0	0.8
3 Small(C)	5	4	8	7	1	0	0	0	0.4
4	5	4	9	8	0	0	0	0	<0.4
5 A+B+C					0	0	0	0	<0.2

*. † and ‡ as in legend of Table 1.

From these results, we conclude that the addition of CHO metaphase chromosomes to senescent cells provides genetic information resulting in the transformation phenotype, and in rescue of senescence. Further support for this view has come from an experiment with fractionated chromosomes. Previously, we showed that metaphase chromosomes could be separated into three size classes in sucrose gradients (large A, medium B, small C), and that individual markers could be localised to specific size classes^{9,10}. Metaphase chromosomes from CHO cells were fractionated in this way, and each fraction was tested for the ability to rescue senescent recipient hamster cells. As may be seen in Table 3, lines 1-3, activity was found in the large size (A) class of chromosomes but few or no colonies were obtained with the other fractions. As before, no colonies were obtained in the absence of chromosomes (line 4) or recipient cells (line 5). Transferent colonies obtained in this experiment were also transformed in phenotype.

Thus, the ability to transform cells behaves similarly to other genetic markers and can be located on a specific size class of chromosomes.

The negative findings with the B and C chromosome size classes provides further evidence that the transformed colonies obtained result from addition of donor chromosomal material, rather than

transformation has been identified. The successful isolation of such transferents also provides evidence that this gene acts dominantly. Our results do not, however, show that this is the only gene involved in the primary transformation step. Further experiments involving a variety of donors, and recipients, will be required to examine questions of this kind.

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Deficient recovery from potentially lethal radiation damage in ataxia telangiectasia and xeroderma pigmentosum

THE enhanced survival that occurs when mammalian cells are maintained in a density-inhibited state for a short time after treatment with X rays, ultraviolet light or drugs has been termed potentially lethal damage repair (PLDR)¹⁻³. It is analogous to liquid-holding recovery in bacteria and yeast, and has been studied using a variety of agents in different cell lines³⁻⁵ and in malignant tumours^{6,7}. To investigate the relationship between this cellular recovery phenomenon and repair at the molecular level, we have examined PLDR in human diploid cell strains with known molecular repair defects. We report here that xeroderma pigmentosum (XP) skin fibroblasts show no PLDR following ultraviolet light irradiation, whereas ataxia telangiectasia (AT) skin fibroblasts are specifically deficient in PLDR following X-ray irradiation. The results suggest that, as in bacterial cells, this cellular recovery phenomenon does reflect molecular DNA repair—probably the excision repair pathway.

XP is an autosomal recessive disease characterised by extreme sensitivity to ultraviolet light and leading to the development of multiple malignant skin tumours. Skin fibroblasts from these patients show a deficiency in the excision repair of ultraviolet light-induced DNA base damage⁸. Five complementation groups designated A to E, as well as a variant group of XP patients, have been identified. AT is an autosomal recessive disease characterised by cerebellar ataxia, telangiectasia of the bulbar conjunctiva and a predisposition to malignant tumours, especially of the reticulo-endothelial system. When treated for malignancy by irradiation, AT patients have in some cases demonstrated a clinical radiosensitivity. Also, skin fibroblasts derived from these patients are unusually sensitive to X rays *in vitro*⁹ and a defect in the repair of gamma irradiation induced DNA damage has been described in some strains¹⁰.

We have examined the capacity of skin fibroblast cell strains derived from patients with XP and AT to perform

PLDR following either ultraviolet light or X-irradiation. The results were compared with those for a normal diploid fibroblast cell strain. PLDR refers to the enhancement in cell survival which occurs in density-inhibited plateau phase cultures when assay for the surviving fraction by subculture at low density is delayed for several hours after irradiation.

The D_0 (inverse of the slope) values of the survival curves following irradiation of these cell strains in exponential growth are shown in Table 1. As previously reported⁹, AT was markedly more sensitive to gamma irradiation than either the normal strain (Li 106) or the XP (A) strain. XP strains A and C, however, were significantly more sensitive to ultraviolet light than either AT or Li 106. Figure 1 shows the results of X-ray PLDR experiments in which Li 106, AT and XP fibroblasts were irradiated in the density-inhibited plateau phase of growth; following X-ray irradiation, cells were subcultured at regular time intervals over 0-24 h to assay for colony forming ability. Doses were chosen to yield approximately the same surviving fraction. Recovery in Li 106 and XP cells is manifested by a maximum increase in survival of 4- and 5-fold, respectively (Fig. 1). In the case of X-ray repair deficient AT cells, however, maximum recovery was by a factor of 1.5, at a survival level considerably lower than for XP or Li 106 (Fig. 1). As the enhanced survival in PLDR is associated with a change in the slope of the survival curve, one would expect a greater increase in PLDR at lower survival fractions².

Figure 2 shows the results of ultraviolet light PLDR experiments in which the normal, XP (group A), and AT cells were irradiated in the density inhibited state, then subcultured 0-32 h later. Recovery in the AT and normal cell strains was approximately 3-fold and 5-fold, respectively, in this experiment (Fig. 2); ultraviolet light-induced PLDR took place more slowly than X-ray PLDR (Fig. 1) as is also the case for molecular (excision) repair following the two types of radiation. There was no recovery in the XP strain; the slight decline in survival seen with short recovery intervals was a consistent finding. Ultimate recovery ratios representing the results of at least three separate experiments for each cell strain following X-irradiation and ultraviolet light are shown in Table 1. No recovery was observed in XP cells from complementation groups A and C following exposure to ultraviolet light, and recovery was significantly reduced in X-irradiated AT cells. Thus, cells which are deficient in the excision repair of ultraviolet light damage did not perform PLDR following ultraviolet light exposure, and X-ray repair deficient cells (AT) seem to have a markedly reduced capacity for PLDR following X-ray exposure. The results with Li 106 are

Table 1 Radiation sensitivity and repair of potentially lethal damage in human diploid fibroblast strains

Cell strain and source	Clinical classification	Cloning efficiency	D_0 (X ray)	Enhancement after X ray	Survival D_0 (ultraviolet light)	Survival enhancement after ultraviolet light
Li 106 (Primary culture)	Normal skin fibroblasts	1.0-6.3%	149±7	4.13±0.03	31±6	4.33±0.34
CRL 1343 (ATCC)	Skin fibroblasts from ataxia telangiectasia	0.7±3.5%	46±3	1.78±0.13	29±3	3.10±0.55
XP12BE (ATCC CRL 1223)	Skin fibroblasts from xeroderma pigmentosum (XP) (group A)	7.9-23.0%	160±17	4.63±1.04	6±1	0.96±0.15
XP8BE (IMR-GM 671)	XP skin fibroblasts (group C)	8.3-16.9%	—	—	8±1	0.91±0.21
XP4BE (ATCC CRL 1162)	XP skin fibroblasts (variant)	0.7-1.2%	—	—	20±2	2.30±0.40

D_0 is inverse of slope of complete survival curve; exponentially growing cells were trypsinised, seeded at appropriate numbers into triplicate 10-cm plastic dishes, and irradiated 18 h later. The D_0 values were derived from least squares linear regression analysis of at least three experiments. PLDR experiments were carried out as described in Figs 1 and 2. Numbers represent mean survival enhancement ± 1 s.e.m. in at least three separate experiments for each cell strain (two experiments for XP (c) and XP variant strains determined after 6 h for X-irradiation and after 24 h for ultraviolet light irradiation).

characteristic of those with five normal human diploid fibroblast strains tested concurrently in our laboratory.

XP variant cell strains show near normal sensitivity to cell killing by ultraviolet light and a normal excision repair capacity, but an apparent deficiency in a post-replication repair process¹¹. The results of ultraviolet light PLDR experiments carried out with an XP variant strain are also shown in Table 1. While groups A and C XP cells were deficient in excision repair, the variant cells were able to carry out PLDR following ultraviolet light irradiation.

We have demonstrated that cells deficient in molecular repair following X-ray (AT) or ultraviolet light (XP) exposure are similarly defective in PLDR. This report is the first to correlate specific DNA repair defects with impairment of a cellular recovery process in mammalian cells. The results suggest that PLDR as a general phenomenon in density-inhibited cells reflects molecular repair processes, which in turn are reflected by an increase in survival. Furthermore, the fact that XP variant cells can perform PLDR while other XP cells cannot suggests that ultraviolet light induced PLDR may specifically reflect the excision repair pathway.

Both AT and XP patients have a predisposition to develop malignancies, and deficiencies in these particular repair systems may be relevant to this susceptibility. Actively proliferating cells in renewal systems may turn over and be lost from the body before they express neoplastic development. However, spontaneous or induced DNA damage in resting stem cells might be repaired mainly by mechanisms such as those reflected by PLDR. These mechanisms appear to act particularly efficiently in non-proliferating cells. Since the stem cell is the likely target as the origin of neoplastic development, deficiencies

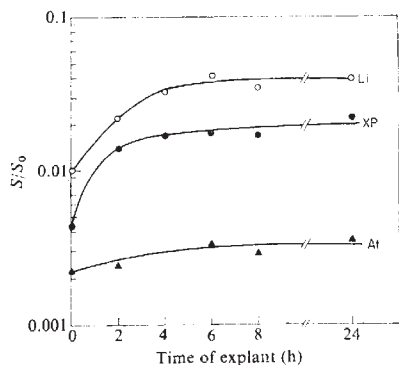


Fig. 1 Repair of X-ray-induced potentially lethal damage ○, Li 106 normal skin fibroblasts ●, CRL 1223, skin fibroblasts from a patient with xeroderma pigmentosum (complementation group A); ▲, CRL 1343, skin fibroblasts from a patient with ataxia telangiectasia. Cultures were maintained at 37 °C in Eagle's minimal essential medium (Gibco F-15) supplemented with 15% foetal calf serum (Microbiological Associates), D-glucose (900 mg l⁻¹), sodium pyruvate (0.66 mg l⁻¹) and chlortetracycline (Aureomycin, Lederle, 50 µg ml⁻¹) in a 5% CO₂ atmosphere. Cells from each strain were studied at similar passage levels in cultures (ranging from the 4th to 28th mean population doubling). Cells were grown to confluency in 6-cm dishes. The culture medium was renewed daily for 3 d after initial confluency and the experiment was performed on the 4th day. Plates were irradiated at room temperature with a 220 kVp GE Maximar unit operated at 15 mA with a dose rate of 80 rad min⁻¹. XP and normal fibroblast cultures were given 700 rad, AT cultures 350 rad. Following irradiation, plates were returned to the incubator. Single plates were removed from the incubator at regular time intervals, trypsinised (0.25% trypsin in Ca²⁺ and Mg²⁺-free Earle's balanced salt solution), and cells seeded in 10-cm dishes in triplicate. The number of cells seeded per dish ranged from 20,000 to 40,000, depending on cell strain and experimental conditions. Medium was changed after 7 d, and after approximately 14 d, cells were fixed, stained with 0.5% methylene blue and scored. Colonies containing 50 or more cells were scored as survivors. The figure depicts a single representative experiment. S, Number of cells surviving at a given time; S₀, number of cells at zero time.

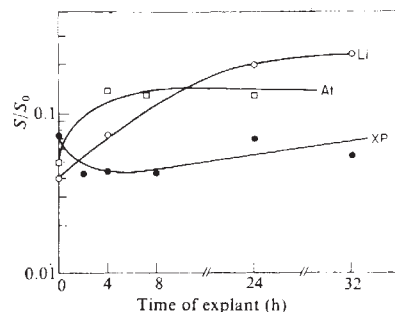


Fig. 2 Repair of ultraviolet light induced potentially lethal damage ○, normal skin fibroblasts; □, AT fibroblasts; ● XP fibroblasts. Cultures were maintained and grown to confluency as described in Fig. 1. On the 4th day, cultures were rinsed with Earle's Balanced salt solution, the solution removed, and the cultures irradiated with a 254 nm light source at a dose-rate of 4.5 erg mm⁻² s. Normal and AT cultures were exposed to 120 erg mm⁻², XP cells 20 erg mm⁻² except XP variant (60 erg mm⁻²). Following irradiation, conditioned medium was added, and the dishes returned to the incubator. Cultures were trypsinised at regular intervals and cells seeded into 10-cm dishes in triplicate. The dishes were maintained, fixed, stained and scored as described in Fig. 1.

in a repair process which is usually thought to be error free (excision-repair) might lead to the accumulation of mutational damage in the DNA and lead to the ultimate expression of malignant change.

We suggest that AT cells are deficient in X-ray PLDR while XP cells are deficient in ultraviolet PLDR, and that this cellular recovery phenomenon reflects molecular repair processes. Deficiencies in this type of repair may contribute to the general predisposition to the development of malignancies in these patients.

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Butyric acid suppression of the *in vitro* neoplastic state of Syrian hamster cells

BUTYRIC acid has been shown to induce morphological and biochemical differentiation in a variety of cells in culture¹⁻⁸. Although the mechanism of action of this short chain fatty acid remains to be understood, changes induced by butyric acid have included: (1) growth inhibition and morphological alterations in HeLa cells, Chinese hamster ovary cells, and neuroblastoma cells²⁻⁴; (2) increases in tyrosine hydroxylase activity and adenylate cyclase activity in neuroblastoma cells^{4,5}; (3) induction of erythroid differentiation in erythroleukaemic cells⁶; and (4) induction of functional β -adrenergic receptors in HeLa cells^{7,8}. We reasoned that the

induction of these differentiated functions might be associated with a concomitant suppression of neoplastic properties. Therefore, we have investigated the effects of butyric acid on the aberrant morphology, anchorage-independent growth, and enhanced fibrinolytic activity of a highly tumorigenic Syrian hamster fibroblast cell line, BP6T (refs 9, 10). We have found that butyric acid causes reversible, specific suppression of each of these altered *in vitro* properties frequently associated with neoplasia. This system could be of value for the investigation of the mechanism of the cellular control of the neoplastic state.

We studied the effects of butyric acid on BP6T cells and two clonal isolates, BP6T-But 1 and BP6T-But 2, derived from BP6T cells. These two clonal strains were obtained by single colony isolation following two subcultures in medium supplemented with 2 mM butyric acid (final pH 7.2), and maintained in this medium unless otherwise stated. The addition of butyric acid to the culture medium produced a slight decrease in the growth rate and cloning efficiency of BP6T cells. Table 1 summarises the growth properties of BP6T cells and the two clonal isolates in unsupplemented medium and in medium containing butyric acid. Butyric acid slowed the doubling time by 4–7 h, and reduced the cell saturation density 20–70%, although the degree of response differed among the three cell strains. These suppressive effects on growth were reversed on removal of the butyric acid (Table 1). Since the tumorigenic BP6T cells exhibit a high plating efficiency on solid surfaces in liquid medium at low cell density, we investigated the effects of butyric acid on this property. BP6T, BP6T-But 1, and BP6T-But 2 cells were reduced in their abilities to form colonies when inoculated at 100 cells per plate in liquid medium containing butyric acid (Table 1). The cloning efficiencies ranged from 14% to 25% in medium containing butyric acid, contrasting with cloning efficiencies of 65–85% in normal medium. Although a diminution of the cloning efficiency resulted from the addition of butyric acid, the efficiency of cloning achieved for all three strains

remained much greater than that obtained with untransformed Syrian hamster embryo cells, which require plating densities of greater than 10^3 cells per 100-mm dish to produce any colonies.

In contrast to the mild effect on the general growth properties mentioned above, butyric acid added to culture medium produced profound effects on the colonial morphology, anchorage-independent growth, and fibrinolytic activity of BP6T cells. The random, criss-crossed orientation of spindle-shaped cells within a BP6T colony grown in the absence of butyric acid (Fig. 1a) is characteristic of Syrian hamster fibroblast lines transformed *in vitro* by chemical carcinogens^{11–14}. Dramatic alterations in this morphology were elicited in BP6T cells by a supplement of 2 mM butyric acid. In medium containing butyric acid, all colonies displayed morphologies similar to BP6T-But 1 and BP6T-But 2 (Fig. 1b and 1c), in that individual cells had a lower nuclear-cytoplasmic ratio and were orientated in a uniform, parallel pattern within the colony. This colony morphology is similar to that of untransformed Syrian hamster cells^{11–14}. To test the reversibility of this change, we plated cells of BP6T-But 1 and BP6T-But 2 into medium lacking butyric acid. In both cases, the transformed morphology of the colonies was restored.

The effect of butyric acid on anchorage-independent growth was examined by cloning in semi-solid agar^{8,12,15}. Following growth for 10 d in medium lacking butyric acid, BP6T cells form colonies at 50% efficiency following suspension of 200 cells⁸. When suspended in agar supplemented by 2 mM butyric acid at concentrations of 10^5 cells per plate, BP6T, BP6T-But 1, and BP6T-But 2 cells were unable to form colonies (Table 1). In contrast to its mild effect on cloning efficiency in liquid medium, butyric acid significantly inhibited anchorage-independent colony formation. To test the stability of the suppressive effect of butyric acid on anchorage-independent growth, the two clonal isolates were grown through 14 passages (approximately 54 population doublings) in liquid medium containing butyric

Table 1 Reversible effect of butyric acid on growth properties of Syrian hamster fibroblasts

Cell type*	Butyric acid concentration (mM)	Population doubling time† (h)	Cell saturation density† (cells cm ⁻² × 10 ⁵)	Cloning efficiency‡ (%)	Anchorage-independent cloning efficiency§ (%)
BP6T	0	13	5.5	85	50 (2 × 10 ⁴)
BP6T	2	20	2.0	15	<0.001 (10 ⁵)
BP6T-But 1	2	20	1.7	14	<0.001 (10 ⁵)
BP6T-But 1	0	15	4.0	65	32 (2 × 10 ⁴)
BP6T-But 2	2	16	4.0	25	<0.001 (10 ⁵)
BP6T-But 2	0	12	5.5	80	40 (2 × 10 ⁴)
Syrian hamster embryo cells	0	14	2.0	2–6	<0.001 (10 ⁵)

*BP6T, a tumorigenic Syrian hamster fibroblast line, was derived from a fibrosarcoma which formed in a newborn Syrian hamster following injection of cells of a benzo(a)pyrene-transformed Syrian hamster fibroblast line. Experiments were conducted with BP6T cells 4–15 *in vitro* passages following tumour isolation. BP6T sublines BP6T-But 1 and BP6T-But 2 were isolated from BP6T cells grown in the presence of an exogenous supplement of butyric acid (2 mM, pH 7.2) for two passages. Colonies which formed following plating of 50 BP6T cells into 100-mm plates containing butyric acid-supplemented medium were isolated and grown continuously in that medium. Syrian hamster embryo cells were derived from 11-d-old embryos; their growth properties and fibrinolytic activities were measured between passages 4 and 8 inclusive. Cell culture procedures used have been described previously^{9,10}.

†The population doubling time was measured during exponential growth at cell densities 1/10 of the cell saturation density for each line. Cell saturation densities were determined in 25 cm² tissue culture flasks (Falcon). For the measurement of population doubling time and cell saturation density in the absence of butyric acid, cells from BP6T-But 1 and BP6T-But 2 were measured one passage following removal of the compound.

‡The cloning efficiency was determined by plating 100 cells into 100-mm × 20-mm (Falcon) optilux polystyrene dishes in 10 ml of medium with or without butyric acid (2 mM). Colonies which formed after 10–14 d were fixed, stained with Giemsa, and counted. The cloning efficiencies are expressed as percentages of the number of cells plated. The cloning efficiency of Syrian hamster embryo cells was determined using 0.5×10^4 – 2×10^4 cells as an initial inoculum.

§Anchorage-independent colony formation was measured by suspending cells in semi-solid agar medium containing 0.4% Difco bacto agar, 0.1% bactopectone, and 10% foetal calf serum dissolved in culture medium⁸. Suspended cells were incubated for 14 d to allow colony growth. The colony-forming efficiencies are expressed as percentages of the total number of cells suspended; the figures in parentheses are the number of cells suspended in each dish.

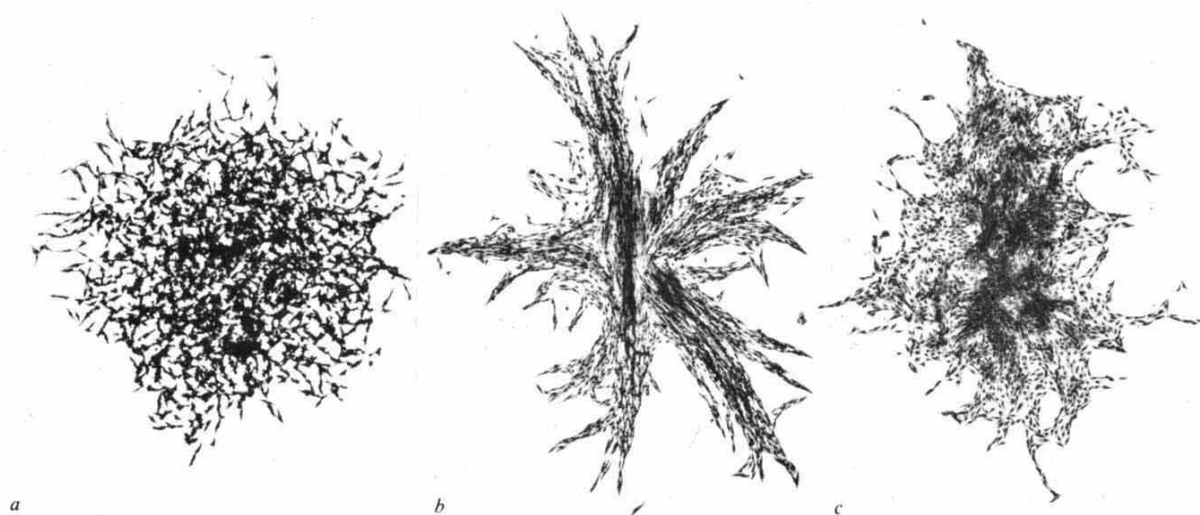


Fig. 1 Effect of butyric acid on colonial morphologies. *a*, BP6T colony formed in the absence of butyric acid, fixed and stained with 10% aqueous Giemsa; *b*, BP6T-But 1 colony (P12) formed in the presence of 2 mM butyric acid; *c*, BP6T-But 2 colony (P 12) formed in the presence of 2 mM butyric acid. All magnifications are $\times 40$.

acid. Following suspension of 10^5 BP6T-But 1 cells in agar containing 2 mM butyric acid, no growth was observed. In contrast, BP6T-But 2 cells partially regained the capacity for anchorage-independent growth, forming 150 colonies following suspension of 2.5×10^4 cells in agar containing 2 mM butyric acid. These results indicate both the stable nature of the suppression of anchorage-independent growth in the presence of butyric acid, and the possibility of obtaining strains which are resistant to this suppressive effect. The suppression of anchorage-independent growth was, however, reversed following removal of the butyric acid supplement. Omission of butyric acid from the culture medium and cultivation of BP6T-But 1 and BP6T-But 2 cells through two passages (approximately eight population doublings) resulted in near restoration of the anchorage-independent cloning efficiency to that of BP6T cells (Table 1).

Previous investigations have indicated that both the variant morphology of transformed lines, and anchorage-independent growth *in vitro* depend on cell-mediated fibrinolytic activity¹⁶⁻¹⁸. Quantitative assays of fibrinolysis have been used to measure the cellular production of the proteolytic factor, plasminogen activator, which cleaves plasminogen to its enzymatically active fibrinolytic form, plasmin^{10,19}. Elevated plasmin-mediated fibrinolysis is a property of BP6T cells and other tumorigenic Syrian hamster cell lines, but not of early passage Syrian hamster embryo cells^{10,16,17}. We examined the effect of butyric acid on both extracellular fibrinolytic activity, present in medium incubated with cells, and on intracellular fibrinolytic activity, present in cell lysates (Table 2 and Fig. 2). Fibrinolytic activity was measured by the extent of degradation of insoluble ^3H -fibrin to soluble ^3H -fibrinopeptides in a cell-free assay¹⁰. BP6T cells exhibited high levels of extracellular fibrinolytic activity, whereas BP6T-But 1 and BP6T-But 2 cells grown in the presence of 2 mM butyric acid produced low levels of extracellular activity, reduced by 85–95% compared with BP6T cells (in the absence of butyric acid) following growth of BP6T-But 1 cells for three passages (approximately 12 population doublings) in medium lacking butyric acid, however, the extracellular activity increased to 40% of the activity of the BP6T cells.

BP6T cells grown for 48 h in medium supplemented with 2 mM butyric acid exhibited an 80–90% reduction in intracellular fibrinolytic activity (Fig. 2). BP6T-But 1 cells grown

in the presence of butyric acid for 14 passages also exhibited reduced intracellular fibrinolytic activity (70–80% reduced compared with BP6T intracellular activity). Following cultivation of BP6T-But 1 cells through three passages in medium lacking butyric acid, the level of intracellular fibrinolytic activity in this strain rose to a level comparable with that of the parent BP6T cells. During this period, the capacity for anchorage-independent growth was restored (Table 1).

Aberrant morphology, anchorage-independent growth, and enhanced proteolytic activity are *in vitro* cellular properties which have been correlated with tumorigenicity.

Fig. 2 Reversible effect of butyric acid on intracellular fibrinolytic activity. The fibrinolytic activity in cell lysates was measured by the method of ^3H -fibrin degradation as described previously¹⁰. (One unit of fibrinolytic activity is as defined in Table 2). Results represent the means of duplicate determinations, following subtraction of radioactivity released by buffer alone (background). ●, BP6T cells; ○, BP6T cells cultured 48 h in the presence of 2 mM butyric acid; □, BP6T-But 1 cells cultured in the presence of 2 mM butyric acid; ■, BP6T-But 1 cells, butyric acid removed for 12 population doublings; ▲, Syrian hamster embryo cells, passage 3.

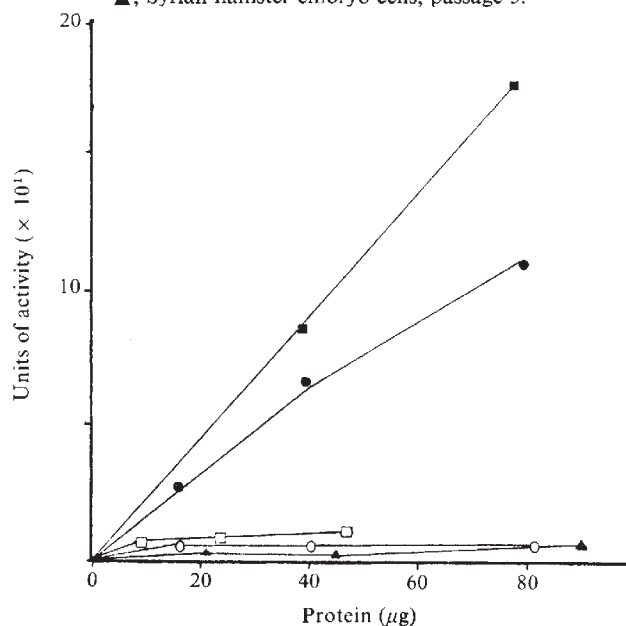


Table 2 Reversible effect of butyric acid on the fibrinolytic activities of Syrian hamster fibroblasts

Cell type	Butyric acid concentration (mM)	Extracellular fibrinolytic activity* (units per 2 ml culture medium)
Control (no cells)	0	3.1
Syrian hamster embryo cells	0	3.2
BP6T	0	42
BP6T-But 2	2	4.2
BP6T-But 1	2	6.1
BP6T-But 1	0	16

*The procedure for measuring extracellular fibrinolytic activity by the technique of ^3H -fibrin degradation has been described¹⁰. One unit of fibrinolytic activity is defined as that which solubilises 10% of the radioactivity release by 100 units of urokinase in 2 h at 37 °C. Values for extracellular activity represent the total units of fibrinolytic factor present in 2 ml of medium cultured in the presence of cells. The background activity observed with control culture medium (which was not incubated with cells) was 3.1 urokinase unit equivalents. The averages of duplicate determinations are reported. Addition of 2 mM butyric acid to medium from BP6T cells cultured in the absence of butyric acid had no significant effect on the extracellular enzyme activity.

The molecular processes, however, which control the expression of these phenotypes are not well understood. One approach toward defining these processes is to regulate the expression of specific phenotypes through the application of exogenous agents. Our results demonstrate that the expression of aberrant colony morphology enhanced fibrinolytic activity, and anchorage-independent growth can be regulated in transformed Syrian hamster cells by the addition of butyric acid to culture medium. Control experiments have shown that butyric acid has no effect on the protein concentration of cell lysates or the activities of two unrelated enzymes, hypoxanthine phosphoribosyltransferase and adenine phosphoribosyltransferase, which are extracted by the same procedure^{20,21} (data not shown). This suggests that the effects we have observed may reflect some degree of specificity of the action of butyric acid. As these phenotypic alterations can be observed during relatively short periods, kinetic analyses of the appearance and disappearance of specific cellular properties following the addition or removal of butyric acid should enable a determination of their interrelationships. An extension of these preliminary studies to an examination of changes in messenger RNA, proteins, and other macromolecules synthesised during the phenotypic reversals, and a determination of the mechanism of action of butyric acid may lead to a better understanding of cellular mechanisms regulating the neoplastic state.

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Actin filaments form the backbone of nemaline myopathy rods

NEMALINE myopathy, a congenital neuromuscular disease, is one of several muscle disorders in which apparently abnormal Z lines, or Z line-type structures emanating from Z lines, has been described^{1–6}. There has been considerable speculation concerning the chemical composition and structural arrangement of proteins in nemaline rods^{2,4,7–12}, but these features have remained unclear: part of this uncertainty is related to lack of understanding of the intact Z line. The only Z line constituent for which there is substantial evidence is α -actinin^{13–16}. We have described the preparation and properties of a Ca^{2+} -activated neutral protease (termed CAF) from muscle that is highly specific in its activity towards myofibrillar proteins and structure^{17,18}. Addition of CAF to isolated myofibrils or to teased muscle fibrils releases undegraded α -actinin from the Z line, with no noticeable effect on myosin, actin, or the remaining myofibrillar structure. We report here the use of CAF as a dissection tool to strip away the dense, amorphous component of the nemaline rods, exposing an underlying set of longitudinal filaments running parallel to the long axis of the original rod. Decoration of these filaments with heavy meromyosin¹⁹ shows that the longitudinal filaments of nemaline rods are composed of actin.

Skeletal muscle containing nemaline rods was obtained by biopsy from a patient with congenital rod disease (second biopsy, case No. 1; see ref. 20 for clinical details) and stored at -60°C until glycerination. It has been shown previously that glyceration does not alter fine structure of the rods^{7,12}. Typical morphology of the muscle consists of areas which contain rods with associated thin filaments interspersed with areas of myofibrils containing moderately widened Z lines (Fig. 1a). Rods are often found grouped together and frequently seem to be branched. There are thin filaments continuous with the longitudinal lines of the rod, as has been described previously^{7,12}. Small numbers of thick filaments usually surround the rod bodies, but are not continuous with it. The longest rod shown in Fig. 1a was 4.3 μm long; many locations in all sections sampled contained rods from ~ 2 to 6 μm long, and all rods demonstrated the five characteristic features described by Engel²¹. In particular, the rods display a set of periodic (140 Å) longitudinal lines running parallel to their long axis; they also show periodic (345 Å) lines perpendicular to their long axis.

A teased, glycerinated nemaline fibre treated extensively with CAF is shown in Fig. 1b. Digestion with CAF removes dense material of the Z line as reported previously; it also removes seemingly dense material from the rods and exposes longitudinal filaments with a uniform diameter (60–70 Å) which span the lengths of the original rods. Thus, their length is dependent on the length of the original rod. Removal of the dense, amorphous material coincides with disappearance of the perpendicular periodicity in the rods. The exposed longitudinal filaments are not as straight as the parallel longitudinal lines present in the original rods, presumably because of loss of supporting material along

their perpendicular axis. As a result, they often can be seen to associate with each other. Stromer *et al.*¹² observed a similar loss of straightness in longitudinal filaments of nemaline rods surviving extended (40 d), low ionic strength extraction.

Because the long, exposed filaments resemble thin filaments in diameter, because thin filaments run into or at least up to the edge of rods in their intact state^{7,12,21} (Fig. 1a), and because of the similarity in resistance to CAF shared by thin filaments¹⁸ and these filaments (Fig.

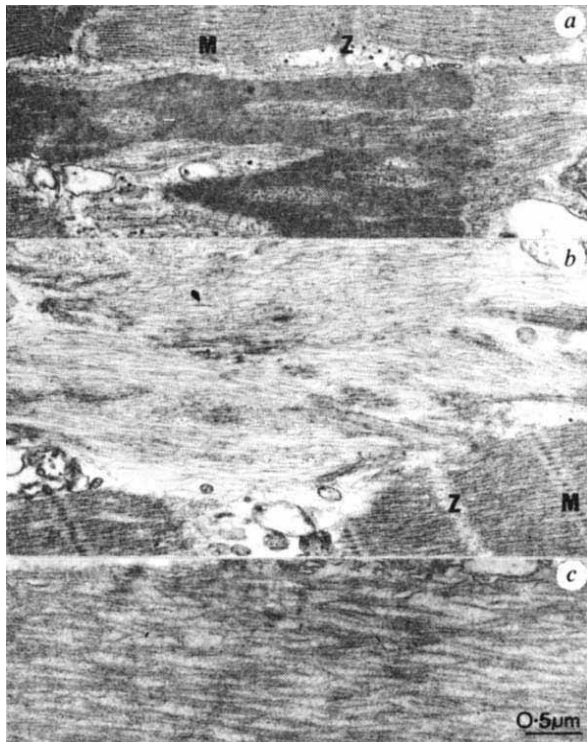


Fig. 1 *a*, Electron micrograph of a longitudinal section of nemaline muscle. Thin strips of muscle were glycerinated as described¹⁹. After thin sectioning, fibres were prefixed with 3% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon-Araldite. Sections were stained with uranyl acetate, followed by lead citrate, and examined in an RCA-EMU-4 microscope operated at 100 kV. Note the large rod body with its long axis parallel to the adjacent myofibril. Longitudinal lines parallel to the long axis of the rod are very evident. M, M line; Z, Z line. *b*, Electron micrograph of a longitudinal section of nemaline muscle after CAF treatment. Teased fibres from glycerinated muscle were incubated with CAF (0.16 mg ml⁻¹) in the presence of 66 mM KCl, 33 mM Tris-acetate, pH 7.5, 3.3 mM CaCl₂, 6.6 mM 2-mercaptoethanol, 0.36 M sucrose, 0.6 mM NaN₃, at 25 °C for 30 min. To ensure complete digestion and removal of amorphous material, the incubation mixture was removed and replaced by fresh CAF mixture four times (30 min each). Following the fifth incubation, the CAF-treated fibres were washed twice in the same solution, except that 10 mM EGTA was substituted for the CaCl₂, and no enzyme was added. Note removal of material in the Z line and appearance of filaments of uniform diameter running throughout the area occupied by the original rod bodies. Small numbers of thick filaments, originally surrounding the rods, also can be seen in scattered clumps in this section. Control experiments (not shown) did not show any morphological changes when CAF was deleted from the incubation mixture. *c*, Electron micrograph of a CAF-treated nemaline sample incubated with heavy meromyosin (HMM). An equal volume of HMM (4 mg ml⁻¹) in 67 mM potassium phosphate buffer, pH 7.0, was mixed with an aliquot of the CAF-treated fibres suspended in the EGTA washing solution and gently stirred for 1 h at 25 °C. Excess HMM was removed by four washes with EGTA washing solution. Note that the long exposed longitudinal filaments bound the HMM to form well known¹⁹ arrowhead structures (compare *b* with *c*). At present it is difficult to determine the direction of polarity of each filament, but analysis of many sections indicates that adjacent filaments often have opposite polarity. Control experiments (not shown) showed no binding of material to filaments when HMM was deleted. Bar: 0.5 μm, photographs ×16,800.

1b), samples of CAF-treated nemaline fibres were incubated with heavy meromyosin. In all cases, the exposed filaments were decorated with the heavy meromyosin forming the well known arrowhead structures similar to those obtained by decorating F-actin filaments¹⁹ (Fig. 1c).

These results show that one component of nemaline rods, the lines running the length of the rod and responsible for the periodicity parallel to the long axis of the intact structure, is composed of actin. Partially exposed filaments that resemble those exposed with CAF can also be seen following extended (up to 40 d) low ionic strength extractions¹², but CAF treatment produces more complete exposure of filaments, does not remove portions of the longitudinal filaments as does low ionic strength extraction, and is much faster. These experiments suggest the usefulness of CAF as a probe of other structures that contain actin filaments. In particular, it would be interesting to use this method to examine the nature of rod bodies and widened Z line-type structures present in various skeletal and cardiac muscle disorders²⁻⁶ to see if they, too, contain an actin backbone.

Based on comparative electron microscope studies of cross sections of Z lines and nemaline rods, MacDonald and Engel⁸ suggested that I (thin) filament profiles are continuous throughout the rods. Engel and Gomez also had suggested earlier that the longitudinal filaments may contain actin because rods disintegrate when I filaments are extracted from the fibre⁷. From our long term selective extraction experiments on nemaline muscle with use of low ionic strength buffers, we concluded that the longitudinal filaments made up the backbone of the nemaline rod, but we were unable to identify their composition clearly¹². Note that our studies reveal only the composition of the longitudinal, filamentous backbone, they do not indicate whether tropomyosin or troponin are on the filaments in the intact rods. Nor do they directly indicate the composition of filaments or material responsible for the periodic lines perpendicular to the long axis of the rods; however, because antibodies to highly purified α -actinin bind to intact rods²² and because of the specificity of CAF in removing α -actinin from Z lines¹⁸, it seems likely that at least some of this material is α -actinin. Although Sugita *et al.*²³ reported that heavy meromyosin would not bind to nemaline rods, their result is not surprising because of the large amount of dense, amorphous material present in the intact rods that is removed by treatment with CAF.

Although it has not yet been determined whether actin terminates at the edge of the Z line or makes up part of the Z line structure in normal muscle, it is interesting that actin filaments are found throughout the rods of nemaline muscle. Stromer *et al.*¹² and MacDonald and Engel⁸ have suggested the rods can be considered to represent a replicating Z lattice or lateral polymer of Z line units. Further studies on nemaline rods may be useful in helping us to understand the Z line structure.

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Does cyclic GMP mediate the slow excitatory synaptic potential in sympathetic ganglia?

THE slow excitatory postsynaptic potential (slow EPSP) of vertebrate sympathetic ganglia is elicited by the muscarinic action of acetylcholine^{1,2}. Its electrogenic mechanism appears strikingly different from that of the well-known (fast) EPSP in many respects—most notably that it is generated with no detectable increase in membrane conductance³. As this type of transmitter action is probably related to cholinergic processes in the brain^{4,5} and possibly in the spinal cord⁶, further study of the slow EPSP in the ganglia as a simpler model of the central cholinergic pathways is of great significance.

It has been proposed that cyclic guanosine monophosphate (cyclic GMP) mediates muscarinic acetylcholine action in various tissues⁷. However, to establish this hypothesis more adequately in sympathetic ganglion cells, it is crucial that the observed postsynaptic depolarisation induced by cyclic GMP⁸ be developed with membrane changes which are identical to those accompanying the slow EPSP itself. We have now used intracellular tests to show that cyclic GMP can elicit two kinds of depolarising response, one of which shows at least some similarity to the slow EPSP.

Intracellular recordings were made in the rabbit superior cervical ganglion at 34 °C, with microelectrodes filled with 1M-KCl, by methods generally the same as those already described³.

Extracellular application of cyclic GMP produced a steady depolarisation, but the accompanying changes in membrane resistance (r_m) were different for different ranges of concentration. (Data are all with dibutyl ester; non-butyryl cyclic GMP behaved similarly but required higher concentrations.) With lower concentrations (25 to 100 μ M) of cyclic GMP, r_m seemed somewhat decreased, with an initial phase of depolarisation that reached a maximum of about 7 mV (Fig. 1, A-a). But this decrease in r_m tended to subside thereafter, whereas the depolarisation was sustained. On the other hand, when the membrane potential was clamped to the original resting level (Fig. 1, A-b), by applying steady polarising current, no decrease in r_m was observed during the action of cyclic GMP. This suggests that cyclic

GMP action itself produced essentially no decrease in r_m , and that the small decrease in r_m during the initial depolarisation of non-polarised cell might be due to a delayed rectification effect (increase in K permeability) produced by the slow depolarisation *per se*. This supposition is better tested by measuring the current-voltage relationship (I-V curve) and comparing the slope of the curves before and during cyclic GMP depolarising action (Fig. 1C). The slope of the I-V curve was found not reduced by cyclic GMP, indicating there was no decrease in r_m . When some flattening of the I-V curves were recorded in the depolarised range, there was rather slight increase in the slope with cyclic GMP. This apparent reduction of the delayed rectification seems to become more marked at 5–10 min after the onset of exposure of the ganglion to cyclic GMP. A similar increase in r_m was reported to occur in single cortical cells of the cat after the intracellular injection of cyclic GMP^{9,10}.

With approximately 10-fold higher concentrations of cyclic GMP, 500–1,000 μ M, the depolarisation was not only distinctly greater (about twofold or more), but it was now accompanied by a substantial decrease in r_m (Fig. 2A) which could not be abolished by repolarising the membrane to the

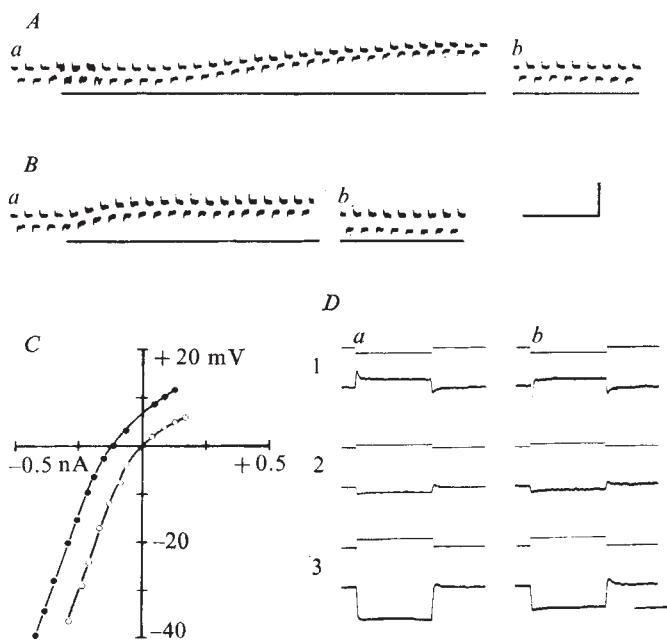


Fig. 1 Changes in membrane potential and resistance in response to lower concentration of cyclic GMP, or to orthodromic nerve stimulation. Resting potential was -51 mV. A-a, response to dibutyl-cyclic GMP at 100μ M (horizontal bar); B-a, slow EPSP elicited by supramaximal preganglionic nerve stimuli at 20/s (bar). (d-Tubocurarine, 20μ g ml⁻¹, was present to block the fast EPSPs¹). Membrane resistance was monitored by the size of recorded voltage pulses resulting from the passage across the membrane of constant current pulses (1 s) at a frequency of 0.5 s⁻¹. Membrane potential was temporarily clamped to the resting level during the course of each depolarisation as seen in A-b and B-b; this was done by passing a steady hyperpolarising current with manual controls. Calibration: 10 mV and 10 s. C, Current-voltage (I-V) relationship obtained from the same cell as in A and B, before (open circles) and 10 min after (filled circles) the application of cyclic GMP (100μ M). A zero level of the ordinate indicates the resting potential without cyclic GMP. D, Electrotonic potentials (lower records of each pair) induced by the current pulses (upper records) of different strengths and polarity. Data correspond to the I-V curves shown in C. Pulses were passed across the membrane at the resting potential level (column a) and at the top of steady depolarisation by 100μ M cyclic GMP (column b). Most of the cells gave 'humped' tonic responses to the passage of weak polarising currents, as seen by Blackman *et al.*¹⁸, so that the potential heights were measured at the steady plateau level when plotted in the graph in C. Calibration: 0.25 nA (hyperpolarising direction upwards) for currents and 25 mV (hyperpolarisation down) for potentials; 0.5 s time-base.

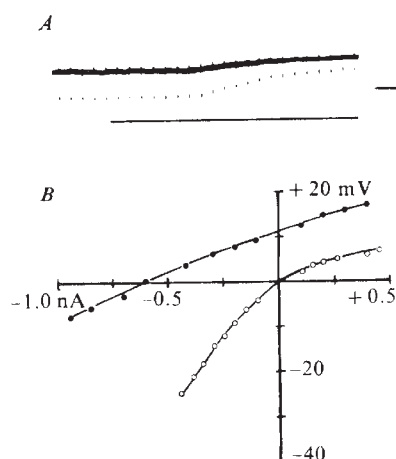


Fig. 2 Changes in membrane potential and resistance in response to higher concentration of cyclic GMP. Resting potential was -53 mV. Frequency of current pulses (200 ms duration in this case) to monitor the membrane resistance was 0.2/s. Calibration: 20 mV and 5 s. *A*, Depolarisation induced by cyclic GMP at $1,000 \mu\text{M}$; *B*, I-V relationships obtained from the same cell, with cyclic GMP (filled circles) and without (open circles).

resting potential level. Further evidence that this marked decrease in r_m is due to the action of cyclic GMP itself is seen in the altered slope of the I-V curve (Fig. 2*B*). The roughly linear portions of the I-V curves near the resting potential indicate a drop in r_m of more than 50% during cyclic GMP action. This second type of depolarising action by stronger cyclic GMP may be a more nonspecific one; it may be related to the responses with increased conductance which can be elicited by either cyclic GMP or cyclic AMP^{11,12}, and to the depolarisation of peripheral nerve by higher concentrations of cyclic GMP⁴.

The difference in r_m effects for low against high cyclic GMP is not simply a quantitatively graded one, related to the magnitude of depolarising action; although the minimum concentration to produce a detectable reduction in r_m appears somewhat variable from cell to cell with the range between 100 – $500 \mu\text{M}$, in a given cell there was always a distinct range of lower concentration in which no detectable change in r_m accompanied a definite depolarisation by cyclic GMP.

During the slow EPSP depolarisation there was little or no change in r_m as reported previously³ (Fig. 1*B*). However, when the membrane potential during the slow EPSP response was temporarily repolarised to the resting level, a definite but small increase in r_m (about 20%) above resting level was usually visible (Fig. 1, *B-b*), (see also refs 3, 5, 6, 13). Actually, determination of full I-V curves in the same cell during muscarinic transmitter action, by acetyl- β -methylcholine (in the presence of *d*-tubocurarine), reveals that (1) in the hyperpolarised range of membrane potential the resistance (slope) is essentially unchanged from that seen for the control I-V curve with no muscarinic action, and (2) in the depolarised range there is a substantial increase in slope, but it only brings the slope back towards though never above that in the hyperpolarised range of control curve. This relationship to r_m is similar to that described for the muscarinic action in nicotinic ganglion cells of the frog¹⁴. Thus the increase in r_m produced by muscarinic acetylcholine in the depolarised range is best explained as an action antagonistic to the delayed rectification effect of depolarisation. This may be due to an action resembling the tetraethylammonium antagonism of delayed rectification¹⁵.

The postsynaptic depolarising action of a low concentration of cyclic GMP was thus found to resemble in its electrogenic nature the slow EPSP in one respect: both show no increase in membrane conductance. Indeed, if elicited in a somewhat hyperpolarised range of membrane potential

levels of -60 to -70 mV, which are probably normal ranges of resting potential of unimpaled cells, the depolarising responses to both muscarinic acetylcholine and the low concentration of cyclic GMP develop with neither increase nor decrease in membrane conductance. On this basis, there is at least some support for the hypothesis^{7,8} that the specific postsynaptic receptor for the slow EPSP response to acetylcholine operates by activating guanylate cyclase, leading to an increase in cellular cyclic GMP, (refs 16, 17, H.K. and N.S.U., unpublished) which in turn induces the subsequent depolarising response. Furthermore, this possibility of the involvement of intracellular cyclic GMP synthesis in the generation of the slow EPSP is in general agreement with our previous observation that the slow EPSP is quite selectively sensitive to the depression of oxidative metabolism^{1,3}.

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Postsynaptic modulation of excitatory process in sympathetic ganglia by cyclic AMP

THE principal neurones of the vertebrate sympathetic ganglia usually respond to suitable preganglionic stimuli with three types of temporally distinguishable postsynaptic potential (PSPs) in the following sequence: an initial excitatory one (fast EPSP), a slow inhibitory one (slow IPSP) and a slow excitatory one (slow EPSP)^{1,2}. These three responses can also be distinguished pharmacologically: the fast EPSP is mediated by a nicotinic acetylcholine receptor, and the slow EPSP by a muscarinic acetylcholine receptor. The synaptic pathway of the slow IPSP includes intervening adrenergic cells (called "SIF" cells³) which release a catecholamine transmitter (identified as dopamine in the case of rabbit^{4,5}), in response to the muscarinic action of acetylcholine, and in turn hyperpolarise the ganglion cells.

It has been found that the brief application of dopamine to the rabbit superior cervical ganglion produces prolonged enhancement of the depolarisation induced by a muscarinic agonist⁶ which can be mimicked by exogenous cyclic adenosine monophosphate (cyclic AMP)⁷. We have now found that iontophoretically injected cyclic AMP into the single ganglion

cell can induce a long-lasting enhancement of the physiologically-elicited slow EPSP. Repetitive stimulation of the preganglionic nerve, in a manner shown to release dopamine from the SIF cells intraganglionically⁴ and to raise ganglionic cyclic AMP^{8,9}, was also found capable of inducing the similar change in the slow EPSP.

Isolated superior cervical ganglia of rabbit were studied at 34 °C, utilising conventional microelectrophysiological techniques¹⁰. For iontophoretic injections, the microelectrode was filled with 0.9M KCl plus 0.1M cyclic AMP (K-salt) and had tip resistance of 40 to 60M Ω . To inject cyclic AMP, 0.5nA was passed for 3 to 10 min.

Following intracellular injection of cyclic AMP, re-tests of the slow EPSP showed enhancement in 4 out of 9 cells tested. A typical example of such an effect is seen in Fig. 1. Amplitudes increased to a maximum after about 15–20 min and then decayed gradually, returning to control levels almost 2 h later. During the enhanced slow EPSPs, there were no appreciable or consistent changes in the partially curarised fast EPSPs responding to the orthodromic volleys; nor were there any significant changes in resting membrane potential or resistance. A second injection of cyclic AMP could renew the enhancement of the slow EPSP (Fig. 1). The absence of effects in some of the nine cells tested could be due partly to the marginally small amounts of cyclic AMP injected in the present method.

Control injections of current alone with a KCl-only micro-electrode in 10 other cells or more produced no such changes in the slow EPSP in the ensuing 2 h. Similarly, with electrodes containing adenosine (non-cyclic) 5'-monophosphate or adenosine triphosphate (ATP) no slow EPSP changes were observed.

A similar change in the slow EPSP could also be induced by the extracellular application of dibutyryl cyclic AMP (1 to 2.5mM added to the chamber bath for 4–8 min) in most of the ganglion cells tested. The absence of any direct hyperpolarising response to dibutyryl cyclic AMP itself, at a concentration which did produce a long-lasting enhancement of the slow

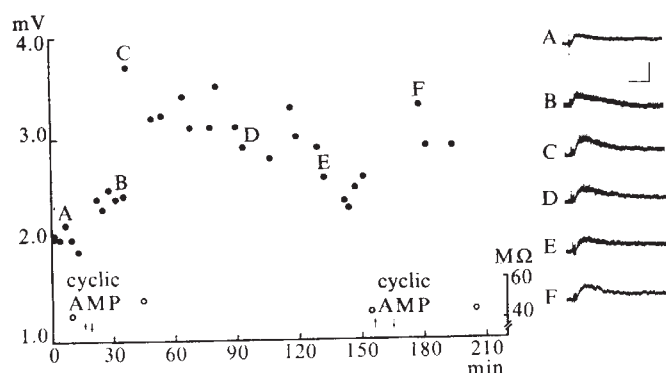


Fig. 1 Slow EPSP amplitude (filled circles) and membrane resistance (open circles) after intracellular injection of cyclic AMP. Current of 0.5nA to inject cyclic AMP iontophoretically was passed between the arrows. Each slow EPSP was evoked at 3- to 10-min intervals by a train of supramaximal preganglionic nerve stimuli (20/s for 1 s), in the presence of d-tubocurarine (20 μ g ml⁻¹) to depress the fast EPSPs. Resting membrane potential of this cell was -50mV. The cell recorded stably for more than 4 h. Inset on the right shows actual oscillograms of the slow EPSPs that correspond to the lettered points on the graph. Stimulation was given at the arrow. Calibration: 5mV and 5 s.

Membrane resistance (ordinate on right side) was measured from the plateau amplitude of the change in membrane potential produced by a constant current hyperpolarising pulse (1 s duration). All exogenous currents were passed through the recording electrode by means of a Wheatstone bridge arrangement. In this and most other experiments with cyclic AMP, the intraganglionically releasable dopamine in the whole ganglion was depleted by a pretreatment with bethanechol⁷, and therefore the slow IPSP were not seen in the oscillograms. This minimises possible effects of dopamine that could be synaptically released during the experimental period, and simplifies the interpretation of changes in the slow EPSPs.

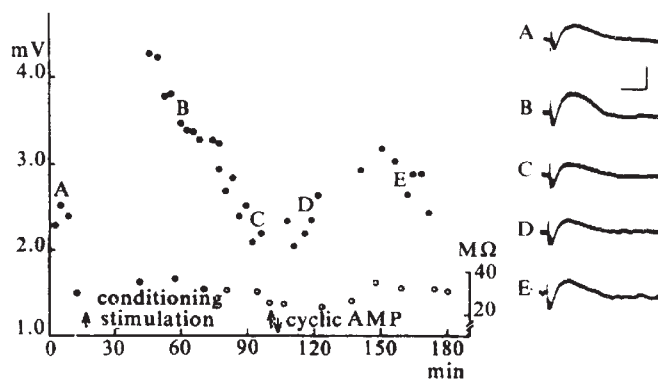


Fig. 2 Slow EPSP and membrane resistance after conditioning stimulation of preganglionic nerve (20/s supramaximal for 1 min, at the arrow) and after a later intracellular injection of cyclic AMP (between arrows). Resting membrane potential was -52mV. Otherwise as in Fig. 1, except that this ganglion was not pretreated with bethanechol, in order that synaptically releasable dopamine remains available. The latter accounts for the normally appearing slow IPSP component before the main slow EPSP in the responses shown by the oscillogram records.

EPSP, is in agreement with other studies using intracellular recordings^{11,12,19}; apparent hyperpolarising responses have been reported with the sucrose-gap technique by some¹³, but not others¹⁴.

Conditioning stimulation with repetitive preganglionic volleys (20/s for 1 min) could also be followed by a period of enhanced slow EPSPs as shown in Fig. 2. The slow EPSPs rose to more than 200% of control value at about 25 min after the conditioning, returning slowly to the original level after about 90 min. The postconditioning modulatory change also occurred without significant changes in resting membrane potential or resistance of the ganglion cell (Fig. 2). In these experiments, post-tetanic potentiation of presynaptic function could contribute to the changes, but this factor was apparently significant only during the initial 10–15 min after conditioning; this could be judged primarily from changes in the fast EPSPs, since presynaptic PTP should presumably affect both the fast and slow EPSPs similarly¹⁵. Furthermore, in some cells there happened to be no detectable slow IPSP, indicating that orthodromic volleys were ineffective in delivering functionally sufficient dopamine by release from the SIF cells⁴; in these cells the conditioning stimulation produced an increase lasting only about 15–20 min in all of the postsynaptic responses, without any longer lasting enhancement in the slow EPSP.

With surface recordings from the whole ganglion similar long-lasting modulatory effects of preganglionic conditioning were observed; in the five ganglia tested, the population slow EPSP response showed enhancement for periods of almost 3 h, with maximal increases of an average of $+60\% \pm 10.2\%$ (range $+39\% - 95\%$). Related long-lasting enhancements of muscarinically-induced postganglionic firing have also been reported to follow a conditioning train of preganglionic volleys, in superior cervical ganglia of the cat *in vivo*¹⁶, and of the rat *in vitro*¹⁷.

Our results provide further support for the proposal of Libet *et al.*⁷ that at least one of the two postsynaptic actions of dopamine, the long-lasting enhancement of the muscarinic depolarising response, may be mediated intracellularly by cyclic AMP. This effect is now demonstrable with slow EPSP responses of the single ganglion cell; it can be produced by intracellularly injected cyclic AMP, eliminating any question of an indirect action of cyclic AMP through a presynaptic release of the other transmitter(s); it is producible through the more physiological route of orthodromic nerve impulses as well as by applied dopamine or cyclic AMP. The absence of significant changes in resting membrane potential or resistance, during enhanced slow EPSP, helps to establish further the effect as an actual modulatory change in the intraneuronal mechanisms

that generate the slow EPSP response to acetylcholine. On the other hand, the absence of a direct hyperpolarising response of the single cell to extracellularly applied dibutyryl cyclic AMP, even at 2.5 mM or more (see also^{11,12}), raises a serious question about the proposed role for cyclic AMP^{13,18} as the mediator of the slow IPSP.

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Ecto-ATPase deficiency in glia of seizure-prone mice

A DEFICIENCY of calcium-stimulated adenosine triphosphatase (Ca^{2+} -ATPase) is seen in brains of mice which are susceptible to audiogenic seizures¹. After studying five other animal models of idiopathic epilepsy we concluded that brain nucleotide metabolism is altered in the epileptic animals and that the lesion might not be confined to Ca^{2+} -ATPase². We speculated that the deficiency of Ca^{2+} -ATPase, which we observed in membrane-enriched brain homogenates, was the expression of incompetent brain cell ecto-ATPases. We have suggested that the genesis of seizures was related to a protracted action of translocated cytoplasmic ATP on cell membranes³ when the latter were deficient in this enzyme. We report here a significant deletion of ecto-ATPase in cultured glia cells raised from neonatal, seizure-prone mice. Between the ages of 20 to 35 d, mice of the DBA/2N strain convulse after repeated exposure to loud noise. Before and after this period, susceptibility to induced convulsions is minimal or absent. Mice of the C57 B1/6N strain are not afflicted in this manner.

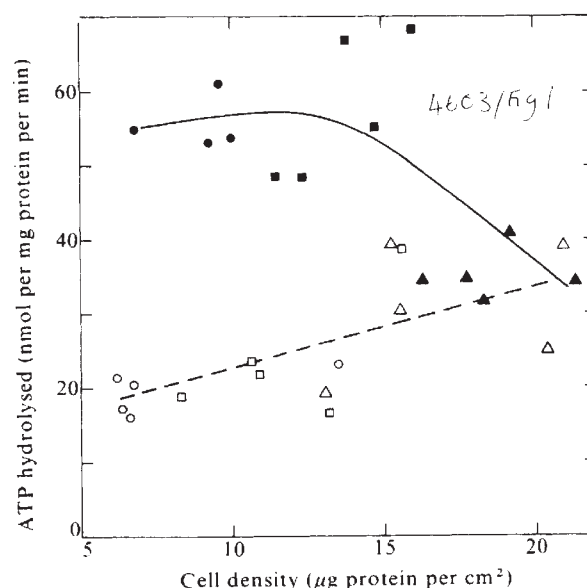


Fig. 1 Ecto-ATPase activity of normal and seizure-prone mouse glia cells. Cells of passage 4 from C57 mouse brains (solid symbols) and from DBA mouse brains (open symbols) were plated out at equal density in 9.6-cm² multi-dish tissue culture trays (Linbro). The cell monolayers were rinsed with 2 ml of modified Dulbecco's medium (mDMEM) and then incubated for 15 min with 2 mM ATP- γ -³²P in mDMEM (see Table 1). Medium was removed after incubation and the cells digested for 24 hours with 1 N NaOH. Protein in the digest was determined according to Lowry *et al.*¹⁰. Each point shown is the average of three determinations. Cultures were assayed at day 7 (●, ○), 11 (■, □) and 18 (▲, △). Curve fitting was by least squares, quadratic polynomials.

Ten separate glia cell cultures (largely of astroblast origin) were obtained from explants of newborn mouse brains according to the procedure of Shein *et al.*⁴. The cultures obtained from neonatal DBA mice (epileptic) are designated D lines and those from the C57 mice (controls), C lines. Experiments were performed on cell cultures of five different D clones and five different C clones after the lines had been propagated through three passages.

Ecto-ATPase assays were performed on intact monolayer cultures which were superfused with substrate in an iso-osmotic medium the composition of which closely resembled the culture medium. The assay of ectoenzyme activity required appropriate conditions and controls (see refs 5, 6). We also determined the activities of ecto-5'-nucleotidase, ecto-ADPase and *p*-nitrophenyl phosphatase (though it is not certain that the latter need is an ectoenzyme). Here, as in our earlier experiments with membrane-enriched brain fractions from DBA, C57 and C3H mice¹ these enzymes served as controls and attested to membrane integrity. The specific activity of some ectoenzymes (mol of substrate metabolised per unit time per mg of protein) can vary greatly with the cell density of cultures or with their proliferative rate⁹. Some of the enzyme assays, therefore, were

Table 1 Conditions for assay of ecto-enzymes

Enzyme	Assay medium	Substrate	Product analysed	Refs
Adenosine-triphosphatase (EC 3.6.1.3)	0.5 ml mDMEM* pH 7.4	2 mM ATP- γ - ³² P	liberated ³² PO ₄	7
Adenosine-diphosphatase (EC 3.1.3.5)	0.5 ml phosphate-free buffer pH 7.4	1 mM ADP	PO ₄ (colorimetric)	8
5'-Nucleotidase (EC 3.1.3.5)	0.5 ml mDMEM pH 7.4	1 mM AM ³² P	liberated ³² PO ₄	7
<i>p</i> -Nitrophenyl-phosphatase (EC 3.1.3.1)	1.0 ml phosphate free buffer pH 7.4	1 mM Tris- <i>p</i> -nitrophenylphosphate	<i>p</i> -nitrophenol (colorimetric)	5

Cells grown to confluency in 9.6-cm² multi-dish trays were rinsed with mDMEM or phosphate-free buffer² before incubation. Monolayers were then incubated for 15–60 min at 37 °C and rotated horizontally at 50 r.p.m. every 90 or 180 s for 6 s.

*Modified Dulbecco's minimum essential medium; it contained neither foetal calf serum nor indicator dyes; Ca^{2+} was 0.5 mM and Mg^{2+} was 5 mM.

Table 2 Ecto-enzyme activity and growth rates of cultured glia cells from seizure-prone (DBA) and control (C57) mice

Enzyme	Day(s)	Culture density ($\mu\text{g protein cm}^{-2}$)	Specific enzyme activity* (nmol substrate hydrolysed per mg protein per min)	
			DBA	C57
Ecto-5'-nucleotidase	7	8.2 $\pm$ 0.9	6.54 $\pm$ 0.80 (15)	7.53 $\pm$ 0.41 (9)
	11	12.7 $\pm$ 0.8	5.13 $\pm$ 0.33 (15)	5.41 $\pm$ 0.44 (15)
	18	18.0 $\pm$ 1.1	2.30 $\pm$ 0.20 (15)	2.56 $\pm$ 0.18 (9)
Ecto-ADPase	4	9.0 $\pm$ 1.0	25.1 $\pm$ 1.52 (15)	20.6 $\pm$ 0.64 (15)
p-Nitrophenyl-phosphatase	4	9.0 $\pm$ 1.0	6.30 $\pm$ 0.26 (12)	10.41 $\pm$ 1.37 (15)
			Growth rate†	
			7-11	1.24 (5)
			11-18	0.70 (5)

Culture conditions were as described in the text and in the legend to Fig. 1. Assay conditions were as for Table 1.

*Values given $\pm$ s.e.m. with number of determinations shown in parentheses.

† $\mu\text{g protein synthesised per cm}^2$ per day.

made at different stages of confluency of the monolayer cultures. Assay conditions are given in Table 1.

When an equal number of D and C cells were plated out, the growth rates (and presumably the viability) of the clones were not significantly different. Both cultures reached about 95% confluency in 11 d. Comparison of the ecto-ATPase activity as a function of cell density revealed a large difference between the two cell lines during the proliferative phases of the cultures (Fig. 1). Ecto-ATPase activity of the glia line derived from seizure-prone DBA mice was significantly lower (Student's *t* test) than that found in C57 glia. On the 7th, 11th and 18th day of culture, D-line glia averaged 35% ($P < 0.001$), 42% ($P < 0.001$) and 86% ($P < 0.3$) respectively of the C-cell controls. During the 7-18 d culture period, ecto-ATPase activity of the C cells had decreased by approximately 35% while that of the D cells had increased nearly 55%. Therefore, on the 18th day of culture, when growth rates were markedly diminished, the ecto-ATPase activity of the epileptic glia line had reached about 85% of that of the controls. This is consistent with the observation that during the later stages of brain maturation in the DBA mouse, the susceptibility of the animal to audiogenic seizures almost vanishes. We have proposed elsewhere that the temporary insufficiency of brain Ca^{2+} -ATPase in the DBA mouse was due to the delayed expression of a more mature ATPase isoenzyme¹. We suggest that this pattern change in ecto-ATPase activity is linked to cell differentiation and (brain) organisation. Although we could not detect organisation in tissue culture, some form of differentiation (for example contact inhibition) may have evoked a similar response pattern in the experiments described here.

Table 2 summarises the values obtained for our presumptive control enzymes. No substantial differences were observed between C57 and DBA glia cells and we assume that the plasma membrane of the DBA glia was normal in other respects. The proposed involvement of glia cells in the genesis of seizures departs from the traditional concept that the epileptogenic lesion ought to be sought in the neuronal elements. Perhaps, because the role of glia in modulating neuronal excitability is poorly understood, their importance in central nervous system function has been underestimated. Several experiments have suggested, however, that neuronal excitability might be mediated by the glia, for instance, by limiting extracellular build-up of substances which might trigger synaptic transmission¹¹. A similar interpretation was made when it was found that neuroleptic drug binding increased with the enrichment of glia cell membranes in brain homogenate fractions¹². Our results do not preclude the existence of neuronal dysfunction in seizure-prone animals, but they strongly suggest that a glial abnormality is implicated in convulsive disorders.

Thus we have shown a deficiency of Ca^{2+} -ATPase, previously demonstrated in seizure-prone animals, to be amplified in the experimental system described here. The ATPase incompetency observed in membrane-enriched homogenates was associated with a lesion in the ectoenzyme activity of the plasma

membrane, and the observed metabolic error (or genetic variant) is a property of the glial elements of the brain (and possibly confined to astrocytes). Our results suggest that a key to the metabolic error in convulsive diseases may be found in the modulating action of ATP on membrane excitability thresholds and the interrelated functions of plasma membrane ectoenzymes.

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D-Glucose inhibits potassium efflux from pancreatic islet cells

ELECTROPHYSIOLOGICAL experiments have shown that D-glucose, the major physiological stimulus of insulin release, produces depolarisation and electrical activity in pancreatic beta cells¹ and that a correlation exists between these electrical changes and the stimulation of insulin release²⁻⁴. Continuous recordings from single beta cells have permitted characterisation of the regular burst pattern of glucose-induced electrical activity⁵; however, the ionic mechanisms underlying these electrical changes remain unclear and both active and passive ionic fluxes may be involved^{6,7}. Islets incubated in a medium containing a very high glucose concentration (20 mM) retain more $^{86}\text{Rb}^{+}$ than do islets incubated in the presence of 3 mM glucose; this suggests⁸ that the depolarising effects of D-glucose on beta cells might be mediated, at least in part, by a decrease in K^{+} permeability. Recent experiments further showed that tetraethylammonium, a specific blocker of K^{+} conductance, potentiated the effect of glucose on both the inhibition of $^{86}\text{Rb}^{+}$ efflux and stimulation of insulin release from isolated islets⁹. The purpose the study described here was to obtain direct evidence for an effect of glucose on potassium efflux from pancreatic islet cells and, by comparing the characteristics of this effect with the changes in insulin release and

electrical activity, gain further insight into the ionic mechanisms involved in the glucose control of the secretory function of the pancreatic beta cells. It will be shown that physiological concentrations of D-glucose, but not of L-glucose, markedly modify K^+ permeability in islet cells, that this effect requires D-

glucose phosphorylation but is not the consequence of insulin release and, finally, that this change in K^+ permeability may account for the initial depolarising effect of the sugar on beta cells.

A perfusion system^{9,10} was used to monitor continuously the dynamics of $^{42}K^+$ efflux from preloaded isolated rat islets. At concentrations of D-glucose below 5 mM, the efflux of $^{42}K^+$ did not follow simple exponential kinetics, as illustrated by the progressive decrease in the $^{42}K^+$ fractional efflux recorded in the continuous presence of 3 mM D-glucose (Fig. 1a). Withdrawal and subsequent reintroduction of the sugar were followed by prompt augmentation and reduction in the efflux rate. An increase of D-glucose to 9 mM markedly diminished and also stabilised the fractional rate of $^{42}K^+$ efflux (Fig. 1a); this effect of high D-glucose was completely reversible on return to the lower concentration of 3 mM. The concomitant changes in insulin release are shown in Fig. 1b. Omission of glucose did not change the basal rate of secretion, whereas stimulation with 9 mM D-glucose elicited the typical biphasic increase in insulin secretion. It should be noted that the first changes in efflux rate and in insulin release were recorded at the same time (3 min after the switch of the stopcock), but that only the changes in release exhibited biphasic kinetics.

The specificity of this effect of D-glucose was tested by comparison with its non-insulinotropic, non-metabolised¹⁴ stereoisomer L-glucose, which does not produce electrical changes in beta cells⁷. In three experiments (not shown), 6 mM L-glucose was added to a perfusate containing 3 mM D-glucose without significantly changing the rate of $^{42}K^+$ efflux as compared with controls maintained in 3 mM D-glucose alone.

The requirement of extracellular calcium for glucose stimulation of insulin release has long been established¹⁵. Figure 2

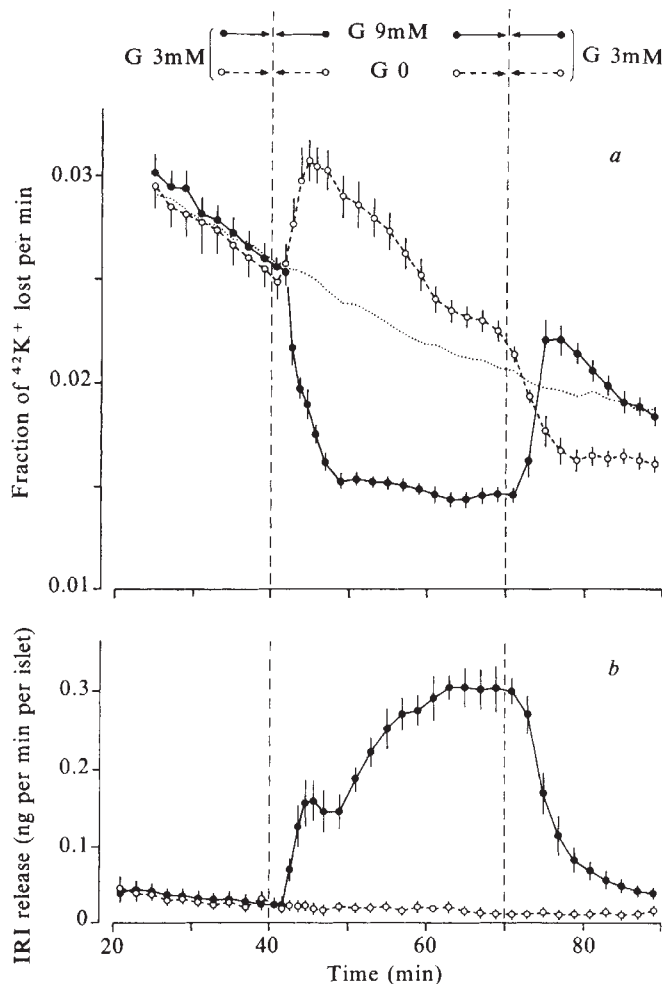


Fig. 1 Effect of D-glucose on potassium efflux (a), and insulin release (b) from perfused rat islets. The perfusate contained 3 mM D-glucose (G) except between min 40 to 70, during which D-glucose was either omitted ($\circ$) or raised to 9 mM ($\bullet$). In a, the stippled line represents control experiments in 3 mM D-glucose throughout. Values are means $\pm$ s.e.m. of 5 experiments. Isolated islets were obtained after collagenase digestion¹¹ of the pancreas of fed male Wistar rats (275–325 g). All experiments were performed at 37 °C with a Krebs–Ringer bicarbonate solution, pH 7.4, containing 6 mM K^+ , supplemented with 0.5% (w/v) bovine serum albumin and equilibrated with $O_2:CO_2$ (94:6). The perfusion system was similar to that previously described¹⁰, but with a smaller chamber (0.3 ml) and a reduced flow rate (1.1 ml min⁻¹). The dead time of the system is 1 min, but no correction was made in the presentation of the results. Effluent fractions were collected at 2-min intervals except for 1-min intervals from min 40 to 46. In efflux experiments, groups of 75–100 islets were first incubated at 37 °C for 150 min, in 1 ml medium containing 3 mM D-glucose and 6 mM ^{42}KCl (IRE, Fleurus, Belgium; 3.5–6.5 mCi mmol⁻¹). They were then washed three times with 5 ml nonradioactive medium at room temperature and transferred to the perfusion chambers. $^{42}K^+$ in the effluent fractions and remaining in the islets at the end of the experiments was counted in a liquid scintillation spectrometer by the Cerenkov radiation, after addition to each sample of 10 ml of a 3-mM aqueous solution of the wavelength shifter 7-amino-1, 3-naphthalene-disulphonic acid¹². After correction for decay, the fractional efflux of $^{42}K^+$ ($^{42}K^+$ released during the time interval/ $^{42}K^+$ remaining in tissue during that time interval) was calculated for each collection interval. In release experiments, groups of 15 islets were first incubated at 37 °C for 150 min in 1 ml medium containing 3 mM D-glucose and then transferred into the perfusion chambers. Immuno-reactive insulin (IRI) was measured in the effluent fractions by a double antibody method¹³ with rat insulin as standard.

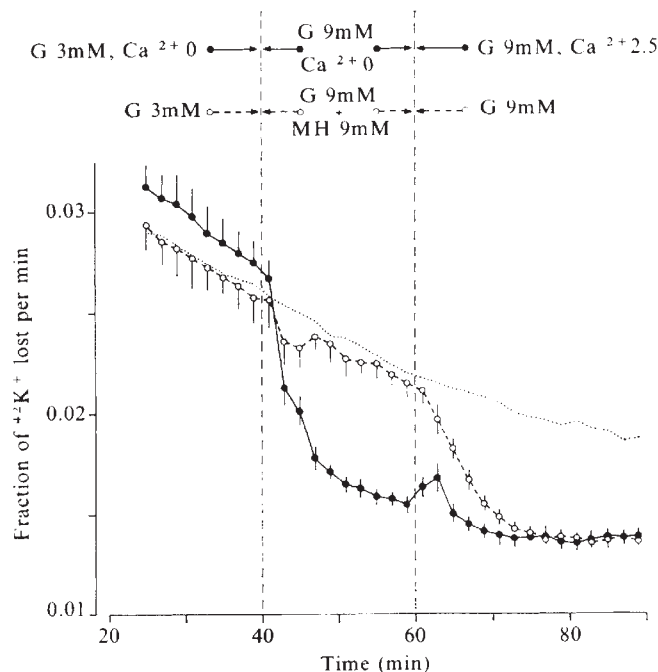


Fig. 2 Effects of the absence of calcium ($\bullet$) or of the presence of mannoheptulose (MH) ($\circ$) on D-glucose induced inhibition of $^{42}K^+$ efflux from perfused rat islets. After incubation with $^{42}K^+$ as described in the legend to Fig. 1, the islets were transferred to the perfusion chambers and effluent fractions were collected at 2-min intervals. D-glucose concentration of the perfusate was 3 mM from the start of the efflux and raised to 9 mM from min 40 onwards. In one series of experiments ($\bullet$), Ca^{2+} was omitted from the medium from min 0 to 60 (total $Ca^{2+} < 25 \mu M$) and added to the medium (2.5 mM) from min 60 to 90. In the other series of experiments ($\circ$), 9 mM mannoheptulose was added to the perfusate during the first 20 min of stimulation with 9 mM D-glucose (min 40 to 60). The stippled line represents control experiments in the presence of 2.5 mM Ca^{2+} and 3 mM D-glucose throughout. Values are means $\pm$ s.e.m. of four experiments.

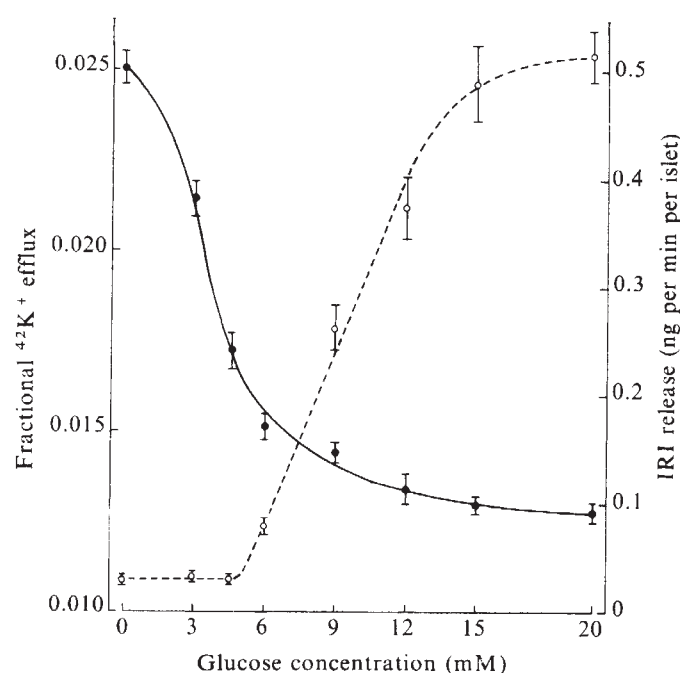


Fig. 3 Effect of different D-glucose concentrations on $^{42}\text{K}^+$ fractional efflux (●) and immunoreactive insulin release (○) from perfused rat islets. After incubation with $^{42}\text{K}^+$ as described in the legend to Fig. 1, the islets were transferred to the perfusion chambers. The fractional release of $^{42}\text{K}^+$ was measured in identical conditions at all concentrations of D-glucose. Each experiment consisted of an initial period of 36 min in the presence of 3 mM D-glucose and a final period of 28 min in the presence of only one of the various glucose concentrations studied. Effluent fractions were collected at 4-min intervals and the fractional release of $^{42}\text{K}^+$ for individual experiments was defined as the average value for the last 16 min of efflux. Values are means $\pm$ s.e.m. of four experiments. The rate of insulin (IRI) release was studied in a similar manner except that in each experiment four alternate D-glucose concentrations were studied in sequence (starting with the lowest concentration). Values are means $\pm$ s.e.m. of six experiments.

shows that the absence of calcium in the medium did not prevent 9 mM D-glucose from reducing the fractional efflux of $^{42}\text{K}^+$ from preloaded islets and thus demonstrates that the changes in efflux are not a mere consequence of the changes in release. Calcium introduction at 9 mM D-glucose elicited a small and very transient increase in K^+ efflux.

Mannoheptulose, which inhibits glucose phosphorylation in pancreatic islets and glucose stimulation of insulin release¹⁴, reversibly prevented the D-glucose-induced reduction in K^+ efflux (Fig. 2). This observation may indicate that glucose has to be metabolised by islet cells to reduce their permeability to K^+ and is in keeping with the report⁷ that mannoheptulose prevents glucose-induced electrical changes in beta cells.

The relationship between D-glucose concentration and the fractional efflux of $^{42}\text{K}^+$ or the rate of insulin release is shown in Fig. 3. The dose-response relationship for secretion displayed the well established sigmoid shape, with a threshold between 4.5 and 6 mM and a half-maximal response around 9 mM D-glucose. In contrast, the major reduction in K^+ efflux rate occurred at low glucose concentrations, below the threshold for stimulation of release. Above 6 mM D-glucose, only minor further changes in efflux rate were recorded, whereas the rate of release increased considerably. The similarity of the glucose dependency curves of glucose metabolism and insulin release has often been reported¹⁴. This does not necessarily imply that the important changes in K^+ permeability observed at the low glucose concentrations are unrelated to glucose metabolism. Thus, the changes in the overall glucose metabolism may not be exactly representative of the changes occurring at all the successive steps of its metabolism; furthermore, it has been recently reported that glucose utilisation and lactate formation

by islets perfused in a system analogous to that used in this study markedly increased at glucose levels which do not stimulate insulin release¹⁵.

Although K^+ efflux was measured from an heterogeneous population of cells, of which beta cells represent only two-thirds, an instructive comparison can be made with electrical recordings obtained directly in beta cells. When glucose is increased to a stimulatory concentration, the membrane potential first decreases and then starts to oscillate in slow waves (bursts) between a depolarised plateau, on to which fast spikes are superimposed, and a more polarised silent level⁵. The duration of the bursts and the frequency of the spikes are both strongly correlated with insulin release and exhibit the same sigmoidal increase as a function of glucose concentration, with a threshold at 5 to 6 mM⁵. In contrast, the membrane potential between the bursts is glucose independent above that threshold. That K^+ permeability diminishes most markedly for changes between 0 and 6 mM D-glucose suggests that the depolarisation between the resting membrane potential and the threshold potential⁵ at which the burst activity (and insulin release) starts is due to the reduction of the beta cell membrane permeability for K^+ . Once the beta cells are sufficiently depolarised, a Ca^{2+} system (as suggested by disappearance of burst and spike activity after Ca^{2+} removal or addition of the Ca^{2+} antagonist, D 600)^{4,7,17} can be activated. The repolarisation at the end of each burst has, tentatively, been explained either by inactivation of the Ca^{2+} transport system or by activation of an electrogenic Na pump^{6,7}. It is also possible that the duration of the bursts (and the magnitude of the concomitant insulin release) is partially controlled by the K^+ permeability of beta cells. This is supported by the further, although small, reduction in K^+ efflux at suprathreshold concentrations of glucose and by the potentiation of insulin release by tetraethylammonium⁹.

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Distribution of Na^+ pump sites in the frog gallbladder

THE energy-dependent transport of fluid across epithelia has been best explained by the three-compartment conception of Curran and MacIntosh¹. This model couples the active 'pumping' of solute with the elaboration of an absorbate of specific tonicity by postulation of local osmosis within the epithelium. This reasoning has been extended in the 'standing-gradient' hypothesis², which accounts for isotonic or hypertonic fluid transport by means of epithelial geometry and the specific transport properties of the cell mem-

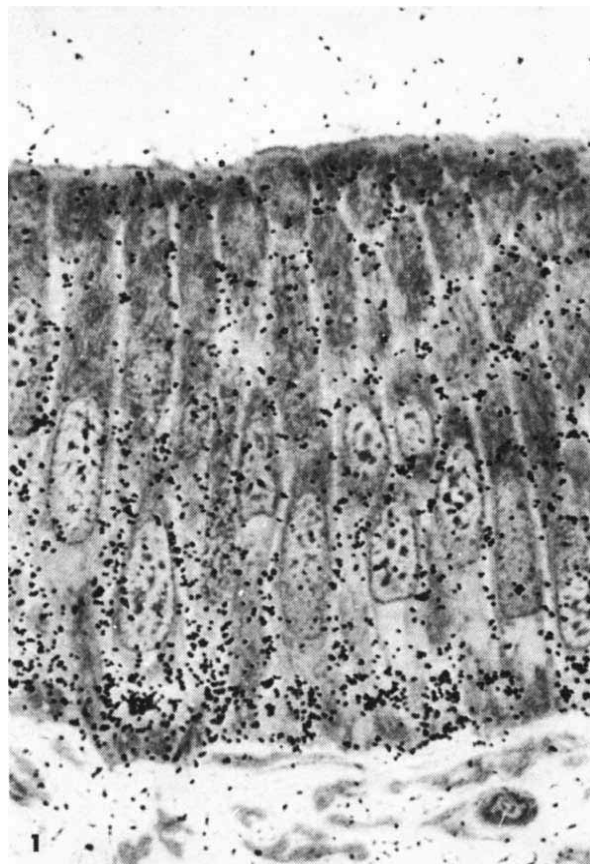


Fig. 1 Radioautograph of the frog gallbladder exposed to $1 \mu\text{M}$ ^3H -ouabain for one hour followed by a 30-min wash period in cold, ouabain-free Ringer's solution. Grains, indicating ^3H -ouabain binding sites, are distributed on the lateral membranes along the whole length of the intercellular space. Note the large number of grains located at the base of the epithelial cells, especially in comparison to the number located near the junctional region between the cells ($\times 1,440$).

brane. The hypothesis depends upon a quantitative assessment of several parameters (membrane hydraulic conductivity, solute-pump distribution and dimensions of epithelial intercellular spaces)³. For example, the model best explains isotonic reabsorption by the gallbladder when it is assumed that the solute-input region (the site of energy-dependent Na^+ pumping) is confined to the apical portion of the lateral intercellular space². To test this important assumption, we studied the distribution of Na^+ pumps in frog gallbladders with radioautography of ^3H -ouabain binding sites⁵. This is a specific procedure for identifying the site of the enzyme, $\text{Na}^+\text{-K}^+$ ATPase^{6,7}, which in this tissue⁴, as well as others, is equated with the Na^+ pump. We find that Na^+ pumps are present only on the basal-lateral surface of the epithelial cells, consistent with their providing solute to the intercellular spaces as the site for local osmosis. However, since the pumps are not limited to the apical portion of the intercellular space, the results do not support the 'standing-gradient' view of isotonic fluid transport.

Gallbladders of the bullfrog, *Rana catesbeiana*, were

dissected free and washed in Na^+ -Ringer's solution⁸ to remove the bile salts. Then the bladders were divided into small pieces (approximately 5–10 mg) and incubated in Ringer's solution in a shaking water bath at room temperature. ^3H -ouabain was added at a final concentration of $1 \mu\text{M}$ and the incubation continued for one hour. This concentration was chosen because it is below the level at which nonspecific binding becomes a major factor⁸. At the end of the incubation period, the vials containing the tissue were drained and refilled with ice cold, ouabain-free Ringer's solution and placed in an ice bath. The medium was changed twice more at 10-min intervals and then the tissue was either quickly blotted and placed in previously weighed vials for wet weight determination or frozen in liquid freon cooled by liquid nitrogen. Frozen pieces of bladder were freeze-dried, fixed and embedded as previously described⁷ except that the tissue was exposed to osmium vapours for three hours to ensure adequate fixation. Sections ($1 \mu\text{m}$) were dried on glass slides, dipped in NTB-2 emulsion (Eastman Kodak) and exposed for 1–6 weeks at 4°C . Quantitative analysis of the grain distribution in each gallbladder was accomplished by a computer-assisted routine similar to the techniques of Mills *et al.*⁷. Hard copies of radioautographs were taped onto a 'spark-pen' data tablet which was serially interfaced to a PDP 11/10 computer and a Princeton 801 graphics terminal. Positional information (the x - y coordinates of field boundaries and grains) was input to the terminal and the computer when the spark-pen was touched to the hard copy overlying the sensitised screen of the data tablet. For each field counted, the epithelial thickness was divided into 10 equal slices beginning at the mucosal border and ending at the basal lamina. In this way, the location of each grain was recorded as its relationship to each slice. The final data were then reported as a histogram of the frequency of grains in each slice.

Figure 1 is a radioautograph of a gallbladder exposed to ^3H -ouabain. Exposed silver grains representing ^3H -ouabain binding sites are associated with the entire length of the intercellular space. The most striking observation is that there is no concentration of grains at the apical end of the intercellular space, as might be predicted, while a large number of grains are preferentially located in the basal one-third of the epithelium. A summary of the grain distribution in gallbladders treated as in Fig. 1 is shown in Table 1. The frequency of grains is fairly uniform for slices 1–7. Slices 8, 9 and 10 show a stepwise increase in the number of grains, with slice 10 having twice as many grains as would be expected for an even distribution.

The observed distribution of grains, representing Na^+ pump sites, may have been due to the inability of ^3H -ouabain to reach the apical end of the intercellular space. This is probably not the case, as increasing the incubation time to two hours, or raising the concentration to $5 \mu\text{M}$, had no apparent effect on the distribution of binding sites. In addition, uptake measurements showed that ^{14}C -inulin reached equilibrium in 10 min, while ^3H -ouabain reached an equilibrium binding value in 15 min. Therefore, the localisation of grains along the lateral intercellular space of the gallbladder is presumed to reflect accurately the distribution of Na^+ pump sites along the whole length of the plasma membrane bordering the space. The concentration of pump

Table 1 Distribution of ^3H -ouabain binding sites in the frog gallbladder

Slices									
1	2	3	4	5	6	7	8	9	10
6.20	7.10	7.23	6.95	7.59	8.05	9.05	12.01	16.10	20.00
± 0.84	± 0.76	± 0.34	± 0.91	± 0.92	± 0.87	± 0.52	± 1.25	± 2.55	± 1.30

Distribution is represented as the percentage of grains ($\pm$ s.e.m.) in each of 10 slices of equal cross-sectional area. Slice 1 begins at the mucosal border and slice 10 ends at the basal lamina. All values corrected for background. ($n = 5$).

sites at the basal end of the cell may be due to the membrane amplification which occurs there⁹; the distribution of pumps may, in fact, be uniform with respect to membrane surface area.

The standing gradient theory would be favoured by a pump distribution which restricted solute input to the apical portion of the intercellular space (the 'dead' end of the channel)¹². Our results, however, indicate that the solute input region is distributed along the entire length of the lateral intercellular space of the gallbladder. This observation, as anticipated and discussed by Diamond and Bossert², does not disprove the standing-gradient hypothesis, but requires the use of extreme values for some of the other important parameters in the model. Isotonicity could still be achieved with a uniform pump distribution if the water permeability of the plasma membranes lining the space was increased upwards. Hill¹⁰, however, has argued that even if the solute pumping region is confined to the upper 10% of the channel length, the osmotic permeability would have to be 3 to 4 orders of magnitude greater than that which has been measured in order to predict an isotonic absorbate by the Segel equation¹¹ for the standing-gradient model. Thus, while the localisation of Na⁺ pumps in the frog gallbladder, as reported here, does not disprove the standing-gradient osmotic flow model, it contributes significantly to the growing body of thought¹² that an alternative explanation for the tonicity of absorbed fluids must be sought.

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Sequences and efficiencies of proposed mRNA terminators

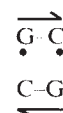
THE base sequences at the 3' end of the different messenger RNA molecules show a pattern of a GC-rich region followed by five to eight Us (refs 1,2). Gilbert's hypothesis¹ that the stability of the template duplex DNA (or of the mRNA–DNA hybrid, or both) determines termination of transcription, implies that the DNA termination sequences, and thus the mRNA sequences at the 3' end, are not unique. Rather, the DNA sequence of the template presumably expresses a certain structural stability, and this pattern of stabilities is responsible for the termination of

transcription. Using these ideas we have found five (but only five) sequences in Φ X174 DNA which have a common pattern of stabilities with previous terminator sequences. These sequences are proposed to be responsible for termination of transcription in Φ X174. Three of the five sequences have five or six As following a GC region instead of the Ts present in known terminators. The lengths of mRNAs predicted on this basis are in excellent agreement with published observations; however, sequence determination of the messengers will be necessary to test our proposal rigorously.

We have analysed those termination sequences given by Gilbert in terms of free energy of formation of each of the Watson–Crick nearest-neighbour sequences. There are some data available from thermodynamic studies of synthetic deoxypolynucleotides³, but we have used the complete set of free energy values determined for RNA double strands⁴. The relative stabilities are

GG > GC > CG > AG ~ TG ~ AC ~ TC > AT ~ TA > AA

For simplicity in representing the sequence in these terms, the nearest neighbours are labelled from 1 to 6 in order of decreasing stability. Note that these numbers characterise a sequence of two base pairs, not a single base pair. For example, GC represents the sequence



The known termination sequences^{1,2} are characterised by a most stable nearest neighbour (GG) followed by two variable terms, then a series of weak nearest neighbours such that at least five of seven of the subsequent terms are 6 on the stability scale. Known termination sequences contain oligo U sequences, but we propose that oligo A sequences are also possible; it is the stability of the oligo dA–dT region which is important.

An analysis of the sequence of the plus strand of Φ X174 DNA (ref. 5) reveals only five locations where this pattern of nearest neighbour stabilities occurs (Fig. 1). There are three promoters^{6,7} *in vitro* and the exact location of these has been determined by Sanger⁵. Using these three known mRNA initiation sites and our five proposed termination sequences, the expected mRNAs may be listed according to their molecular weights and which protein codes they contain. A comparison between this prediction and the mRNA found *in vivo*⁸ is given in Table 1. In general, excellent agreement is found.

Hayashi *et al.*⁸ separated fourteen peaks of different molecular weight mRNAs by electrophoresis. By hybridisation of these fractions with restriction fragments, those authors were able to orient nine mRNA fractions on the Φ X174 genome. All were accounted for by predicted mRNAs; seven showed exact agreement in both size and content (fractions 0 and IX are less certain).

Hayashi *et al.*⁸ reported that RNAs starting at promoter A' are rapidly degraded *in vivo*, with the possible exception of RNA species I. In Table 1, the predicted RNAs which do not have counterparts *in vivo* are those that initiate at promoter A'; and the observed fractions *in vivo* which do not correspond to predicted messengers are of smaller molecular weight and thus may represent degradation products.

Of particular significance is the observation that for each of four predicted terminators, one or more observed mRNA fractions may be assigned. No additional terminators need be proposed to explain the observed data. None of the predicted mRNAs which are terminated by T₁ is observed experimentally; therefore, they are not shown in Table 1. Since T₁ differs from the other predicted terminators by the presence of a guanine residue interrupting the AT-rich region, and thus contributing two stronger stability terms, it may be expected to be a less efficient terminator. Axelrod⁷, however, has observed a rho-dependent terminator *in vitro* which maps at a location in agreement with our predicted T₁.

Terminator*	Position†	Sequence and Stability Terms	
T ₄	3961	G G A G G C	T T T T T T A T G G T T
		1 4 4 1 2 4	6 6 6 6 5 5 4 1 4 6
T _H	2635	C G A C C C	T A A A T T T T T T C C
		3 4 4 1 1 4	5 6 6 5 6 6 6 6 6 4 1
T ₂	854	A A A G G T	A A A A A C G T T C T
		6 6 4 1 4 5	6 6 6 6 6 4 3 4 6 4 4
T _G	2308	A G C G G T	A A A A A T T T T A A T
		4 2 3 1 4 5	6 6 6 6 5 6 6 6 5 6 5
T ₁	4429	T A C C C C	A A A A A G A A A G G T
		5 4 1 1 1 4	6 6 6 6 4 4 6 6 4 1 4

Fig. 1 Proposed terminator sequences and stability terms for ΦX174. The approximate location of the last residue transcribed is before the right-hand solid line, except for terminator T_H (indicated by the dashed line).

* Notation from ref. 7: T_G and T_H refer to terminators labelled "start of gene G" and "start of gene H", respectively. † Indicates location of first base listed, using numbering of ref. 5.

Since Hayashi *et al.*⁸ have given values for the relative number of molecules of each of the fractions mapped, the efficiencies of each predicted terminator may be calculated. This calculation uses only those fractions assigned to starts at promoter A. The results in Table 2 are consistent with Axelrod's *in vitro* obser-

their primary sequence. The terminator sequences are listed in order of decreasing efficiency in Fig. 1. All terminators require a strong nearest-neighbour interaction, outlined in the box at the left. The other boxed region is composed of ten nucleotides. The efficiency of termination seems to be correlated with the number

Table 1 Comparison of mRNAs found by prediction and by experiment

Fraction	<i>In vivo</i> *		Prediction		Promoter Terminator†
	Mol. wt × 10 ⁻⁶	Proteins	Mol. wt × 10 ⁻⁶	Proteins	
0	2.3	(C) DEFGHABCDE	2.61 2.29 2.00	ABCDEJFGHABCDE BCDEJFGHABCDE DEJFGHABCDE	P _A /T ₂ P _A /T ₂ P _G /T ₂
I	1.8	ABCDEF GH	1.83	ABCDEF GH	P _A /T ₄
II	1.5	BCDEF GH	1.52 1.38 1.27	BCDEF GH ABCDEFJ(G) ABCDEFJ	P _A /T ₄ P _A /T _H P _A /T _G
III	1.3	(C)DEFGH	1.23	DEJFGH	P _G /T ₄
IV	1.1	BCDEF G	1.07	BCDEFJ(G)	P _A /T _H
V	0.98	BCDEF	0.96	BCDEFJ	P _A /T _G
VI	0.82	(C)DEF	0.79 0.78	DEJF(G) ABCDE	P _G /T _H P _A /T ₂
VII	0.73	?			
VIII	0.66	?	0.67	DEJF	P _G /T _G
IX	0.6	BCDE	0.46	BCDE	P _A /T ₂
X	0.52	?			
XI	0.43	?			
XII	0.32	?			
XIII	0.23	D(E)	0.17	DE	P _G /T ₂

*From ref. 8.

†Notation as in ref. 7.

vation that T₄ is a strong terminator, whereas T₂ is less effective. In addition, the terminator designated T_H is seen to have a similar efficiency to T₂, and T_G is even less effective.

The relative efficiencies of the terminators may be related to

of adjacent T or A residues and their distance from the strong nearest-neighbour interaction. Thus, T₆ starting in the first position outlined is more efficient than A₆ in the same place or T₆ starting further to the right. All of these are more efficient than A₅

Table 2 Efficiency of terminators

Promoter	Terminator	Fraction	Relative no. of molecules	Terminator efficiency	Sequence
P _A	T ₂	0	0.3	—	GGCTTTTTTATGG
P _A	T ₄	II	2.2	79	CCCTAAATTTTTT
P _A	T _H	IV	3.1	53	GGTAAAAAACGTT
P _A	T ₂	IX	9.5	50	GGTAAAAATTTTA
P _A	T _G	V	3.0	34	
Relative no. of molecules					
			Observed	Predicted	
P _G	T ₂	0	0.3	0.3	
P _G	T ₄	III	2.3	2.1	
P _G	T _H	VI	5.2	2.9	
P _G	T ₂	XIII	9.0	(9.0)	
P _G	T _G	—	—	2.8	

starting in the first position. Given our assumptions about the importance of weak nearest-neighbour interactions in this region, it seems reasonable to speculate that the presence of G after A₅ in T₁ may make this a much weaker, or totally ineffective, terminator. Yet in view of the *in vitro* evidence, this may act effectively in the presence of rho factor. There is obviously still much to be learned about terminator efficiency and sequence, but here we stress the importance of duplex stability (DNA-DNA and DNA-RNA).

On the basis of terminator efficiencies calculated from promoter A, the relative number of molecules for each terminator for another promoter can be calculated, given the assignment of any one mRNA for that promoter. Thus for promoter G, the distribution of RNAs may be calculated given the assignment of RNA XIII to T₂. Good agreement is found for fraction O and III, but one finds that fraction VI contains nearly twice as many molecules as expected. This may be explained by reference to Table 1, where it is seen that two mRNAs of the same molecular weight, but arising from different promoters and terminators, have probably been collected together as fraction VI. Determination of the base sequences of the mRNAs can verify the proposed terminators.

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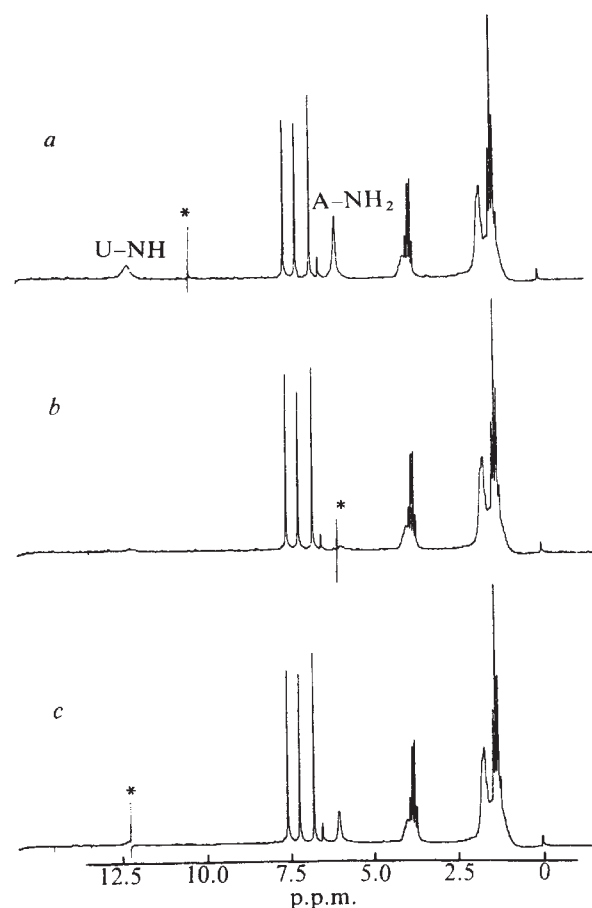


Fig. 1 ^1H NMR spectra of a mixture of 9-ethyladenine and 1-cyclohexyl-5-bromouracil (both at 0.2 M) in chloroform. *a*, Irradiated at the position where is no signal. *b*, Irradiated at the amino proton of 9-ethyladenine. *c*, Irradiated at the imino proton of 1-cyclohexyl-5-bromouracil. Asterisk indicates the irradiated position.

Direct proton exchange between complementary nucleic acid bases

SPECIFIC interaction between complementary nucleic acid bases, in which adenine forms strong hydrogen bonds specifically with thymine (uracil) and guanine with cytosine, is believed to be the molecular basis for the transfer of genetic information. Infrared spectroscopy and nuclear magnetic resonance have shown that such specific interaction is intrinsic to nucleic acid bases¹⁻⁵. In spite of much knowledge about the static structure of the interaction⁶⁻⁹, little is known about the dynamic property of the base pairs. We report here quantitative evidence of direct proton exchange between complementary nucleic acid base derivatives.

9-Ethyladenine (9EA) and 1-cyclohexyl-5-bromouracil (5BrU) can form dimers in chloroform solution³. Proton magnetic resonance spectra of a 9EA-5BrU (1:1) mixture are shown in Fig. 1. When the amino proton signal of 9EA was irradiated, the imino proton signal of 5BrU disappeared. Similarly irradiation at the imino proton of 5BrU reduced

the intensity of the amino proton of 9EA. With dimethylsulphoxide (DMSO) solution of the 9EA-5BrU (1:1) mixture, there was a smaller decrease in peak intensity than with chloroform solution. DMSO more effectively disturbs the formation of solute-solute hydrogen bonds. Thus the observed decrease of the nonirradiated proton signal correlates closely with the quantity of hydrogen bonded dimers between 9EA and 5BrU.

These results can be explained as follows. Protons are reversibly exchanging between the amino group of 9EA and the imino group of 5BrU, and thus the irradiated proton moves to the nonirradiated site through the formation of hydrogen bonded dimers. The exchange of saturated protons produces partial saturation of the nonirradiated proton site to weaken the intensity of the nonirradiated proton resonance, if the exchange rate is faster than the relaxation time^{10,11}. Irradiation at the signal of water proton contaminated in solution gives little change in the intensity of other proton resonances. This suggests that most protons do not exchange by way of the water in the solvents.

The same experiment was performed using a mixture of 9-ethylguanine (9EG) and 1-methylcytosine (1MC) in DMSO. In this system it is interesting that proton exchange occurs between the imino proton of 9EG and the amino proton of 1MC (Fig. 2), but the amino proton of 9EG does not exchange with both the amino proton of 1MC and the imino proton of 9EG (Table 1). If the formation of cyclic hydrogen bonds of the G-C pair^{8,9} is a sufficient condition for the exchange of protons, the amino proton of 9EG should ex-

Table 1 Partial saturation induced by intermolecular exchange of saturated protons

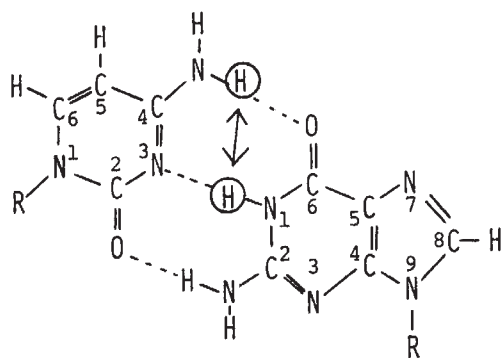
Base pairs	Solvent	Irradiated nuclei	Decrease in the peak intensity (%)				U-imino
			G-amino	C-amino	A-amino	G-imino	
9-ethyladenine + 1-cyclohexyl-5-bromouracil*	CDCl ₃	A-amino U-imino H ₂ O			36 0		100 0
	DMSO	A-amino U-imino H ₂ O			17 0		40 0
9-ethylguanine + 1-methyl-cytosine†	DMSO	G-amino		12		20	
		C-amino	12			86	
		G-imino	0	71			
		H ₂ O	3	5		20	

*, 0.2 M.

†, 0.1 M.

change with the other protons participating in hydrogen bonds, in view of the distance between the protons. These results make us consider factors other than distance for the mechanism of intermolecular proton exchange. The proton transfer induced by the imino and enol tautomers¹² or protonated species¹³ can be considered as possible explanations.

For this we shall estimate quantitatively the life time of the proton exchange. We consider the system where the nucleus X exchanges between site A and site B, and the nucleus X in the site B is saturated by irradiation at the A site proton. Forsén and Hoffman's analysis^{9,10} gives 0.1 s as the life time for the proton exchange if we use the data of the spin lattice relaxation times observed separately (0.23 s for A-NH₂). Thus the proton exchanges 10 times in a second for the 9EA-5BrU system in chloroform.

**Fig. 2** Exchangeable protons in the G-C pair.

Here we have used model compounds for nucleosides, and our experiments were carried out only for the monomer systems. In spite of these limitations, there is no reason to deny proton exchange in nucleic acid. The structures of nucleic acid bases which are realised at the time of the proton exchange are different from the normal structures. Although we do not know the real effect of proton exchange in complementary base pairs on biological process, such altered structures might induce mispairing in the replication and transcription of nucleic acids¹⁴.

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Relaxin and its structural relationship to insulin

THE sequence of the ovarian peptide, relaxin, has recently been reported^{1,2} and the observation¹ that it can be accommodated into the insulin fold has been discussed^{3,4}. Only 11 residues, including the cystines, are common to insulin and relaxin (Fig. 1), but the probable identity of the cystine pairings and the preservation of the hydrophobic character of buried residues suggests some structural homology between the two hormones. The extent of sequence changes and the remote relationship between the corpus luteum and the pancreas, the respective sources of relaxin and insulin, makes homology of the two hormones most interesting. We have therefore rigorously examined the relaxin conformation by a computer graphics system and found it possible to accommodate the relaxin sequence within the insulin main-chain geometry.

We first considered the packing of residues whose equivalents in insulin are buried or partly buried. We chose molecule 2 of the 2-zinc insulin⁵ structure for the comparison since the conformations in this monomer are most closely related to those of 4-zinc pig insulin⁶ and the dimeric hagfish insulin (Cutfield, J. F. *et al.*, in preparation). The atomic positions of this insulin molecule are now accurately known and for the buried residues the estimated errors are 0.1-0.2 Å. Using Labquip models, we found that the internal hydrophobic residues of insulin could be replaced by the equivalent relaxin residues without the apparent need to adjust the main-chain conformation. In particular, there was an extensive cavity into which tryptophan B₁₃ seemed to fit nicely. Because of the limitations and inaccuracies in model building which we consider might be misleading, we then used a computer graphics system^{7,8} to superimpose the relaxin chains on to the insulin backbone. Any short contacts were indicated immediately by dotted lines between the offending atoms. Side-chain conformation were adjusted by rotations about single bonds until acceptable stereochemistry and packing was achieved. We found that tryptophan B₁₃ can indeed be directed into the relaxin core with no contacts unacceptable in relation to the precision of the insulin coordinates. This and the equivalent insulin structure are shown in Figs 2b and 2a.

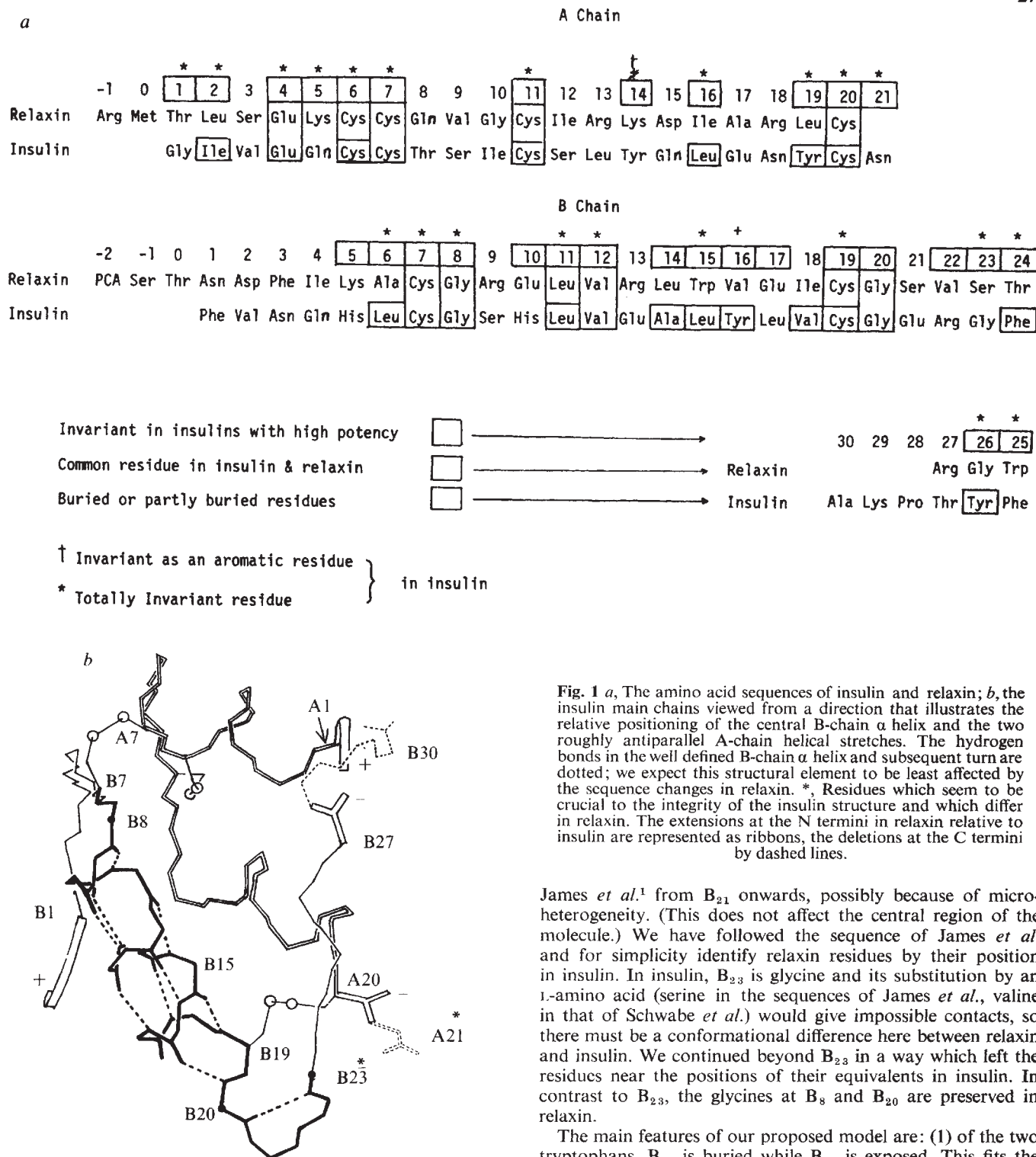


Fig. 1 *a*, The amino acid sequences of insulin and relaxin; *b*, the insulin main chains viewed from a direction that illustrates the relative positioning of the central B-chain α helix and the two roughly antiparallel A-chain helical stretches. The hydrogen bonds in the well defined B-chain α helix and subsequent turn are dotted; we expect this structural element to be least affected by the sequence changes in relaxin. *, Residues which seem to be crucial to the integrity of the insulin structure and which differ in relaxin. The extensions at the N termini in relaxin relative to insulin are represented as ribbons, the deletions at the C termini by dashed lines.

Some alternative arrangements of buried side-chains are possible without adjustment of the main chain. An advantage of the computer graphics system is that such alternatives can be rapidly explored. In particular, we found that tryptophan B₁₅ can be orientated in a second distinct way (Fig. 2c) related principally by 180° rotation about the C _{β} -C _{γ} bond, and corresponding essentially to that reported by Bedarker *et al.*⁴. It gives a rather more open hydrophobic core than our preferred alternative of Fig. 2b.

We built the terminal stretches of chain beyond the disulphide bridges as they occur in insulin. The additional residues at the N termini were built in arbitrary but sensible conformations giving reasonable contacts to the main body of the molecule. There were two difficulties at the B-chain C-terminus. The sequence reported by Schwabe *et al.*² differs from that of

James *et al.*¹ from B₂₁ onwards, possibly because of micro-heterogeneity. (This does not affect the central region of the molecule.) We have followed the sequence of James *et al.* and for simplicity identify relaxin residues by their position in insulin. In insulin, B₂₃ is glycine and its substitution by an α -amino acid (serine in the sequences of James *et al.*, valine in that of Schwabe *et al.*) would give impossible contacts, so there must be a conformational difference here between relaxin and insulin. We continued beyond B₂₃ in a way which left the residues near the positions of their equivalents in insulin. In contrast to B₂₃, the glycines at B₈ and B₂₀ are preserved in relaxin.

The main features of our proposed model are: (1) of the two tryptophans, B₁₅ is buried while B₅ is exposed. This fits the chemical and fluorescence observations of Schwabe and Braddon⁹, who found that one tryptophan reacted readily without altering the hormone's potency, while reaction of the second was very difficult and inactivated the hormone; (2) there are compensating changes in side chains that are spatially related, such as B₆ and B₁₄ and A₃ and A₁₆, which are consistent with insulin and relaxin having the same internal organisation¹¹ (see Fig. 1a); (3) there is complementary distribution of the many charged side chains, particularly noticeable on the helical stretches. This not only supports the proposed folding but also suggests that the relaxin molecule achieves stability as a monomer through these favourable interactions¹⁰; (4) detailed changes near B₂₃ modify the characteristics of the monomer surface involved in dimer formation in insulin; (5) residues B₉ to B₁₉ fit well in an unbroken α helix, so that the

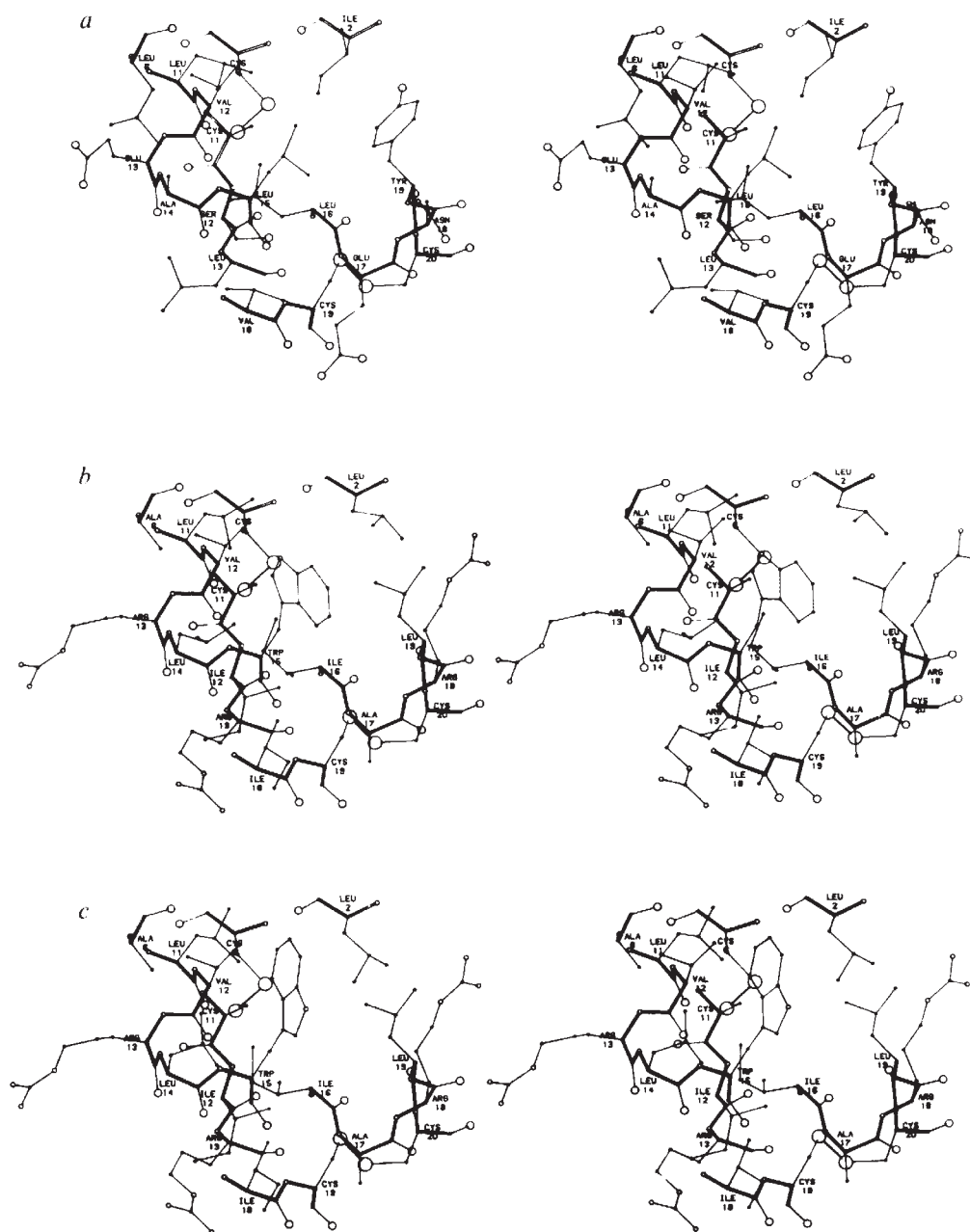


Fig. 2 Buried residues in the central core: *a*, 2-zinc insulin; *b* and *c*, alternative arrangements of Trp B₁₅ in relaxin. The A chain is shown by open bonds and the B-chain by solid bonds.

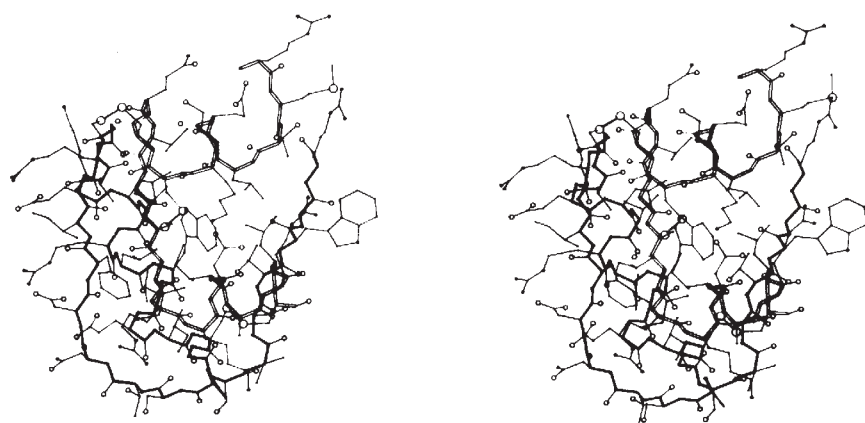


Fig. 3 A stereo view of the complete relaxin molecule with B₁₅ as in Fig. 2*b*.

improvement in sequence homology achieved by displacing the two sequences at B₁₄ to B₁₇ (ref. 1) seems to have no significance in structural terms.

We consider that relaxin has a three-dimensional organisation similar to that of insulin. We do not yet know the closeness of this relationship but note that insulin itself shows considerable structural variation⁶. Thus, while the B-chain α helix is closely preserved, we find widely different conformations of the terminal residues and some alterations of the A-chain helical structure. Therefore, we might expect greatest similarity between relaxin and insulin in the α -helical B-chain and increasing differences in the A chain and the chain termini.

Amongst the many sequence differences between insulin and relaxin, there are two of particular relevance to function. The first is the absence of residue A₂₁ in relaxin. When A₂₁ asparagine is removed from insulin so that, like relaxin, its A-chain terminates at A₂₀ cystine, the hormone's solution and spectral properties are profoundly altered¹¹, its A-chain is apparently perturbed¹² and its biological potency is much reduced^{11,13}. Second, the substitution of B₂₃ glycine by an L-amino acid has been shown by the Chinese studies effectively to abolish insulin's biological activity¹⁵. In diverging from insulin at A₂₁ and B₂₃, relaxin therefore will be without the capacity to behave like insulin. (We note that in the insulin-like peptide responsible for the non-suppressible insulin-like activity in the serum (insulin-like growth factor), B₂₃ is glycine and A₂₁ is alanine, though this is not the C-terminal amino acid¹⁶).

X-ray analysis of relaxin and other hormones with recognised homology to insulin¹⁷ could clarify the relationships between their sequences, structures and biological behaviour.

We thank Professor T. L. Blundell for showing us the paper by Bedarkar *et al.* before publication. The coordinates of our model have been deposited with the Protein Data Bank.

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A novel continuous sequence of 41 aspartic and glutamic residues in a non-histone chromosomal protein

THE structural functions of four of the five histones are now partially understood¹ and the nucleosome structure of chromatin well established²⁻⁴. However, superimposed on this basic nucleosome structure is some heterogeneity with respect to the other chromosomal proteins, since although it has been shown that isolated nucleosomes contain non-histone chromosomal proteins^{5,6}, there are insufficient of some classes for one per nucleosome. These nucleosomal non-histone chromosomal proteins have been designated the high-mobility group (HMG) proteins. They have been fractionated, and the main components, HMG 1, HMG 2, HMG 14 and HMG 17 have been isolated in a pure form⁷⁻⁹. The complete amino acid sequence of one of these proteins, HMG 17, has been determined¹⁰. We report here that the C-terminal region of protein HMG 1 contains an extremely unusual sequence of amino acids—41 aspartic and glutamic acid residues.

The molecular weight of HMG 1 as determined by sedimentation equilibrium, is approximately 26,000. One of the interesting features of HMG 1 is the fact that over 50% of its amino acid residues are charged. Like the histones, 25% of the residues are basic. However, unlike the histones this protein also contains 30% acidic amino acids. Because of the quantities present in the nucleus (approximately 10⁶ molecules), we feel that, like the histones, HMG 1 is a structural protein and is probably not involved in specific gene control.

Previous work has shown that the acidic amino acids in HMG 1 are irregularly distributed within the molecule¹¹. A cyanogen bromide peptide, CB2, approximately 120 amino acid residues in length, has been isolated from HMG 1 (ref. 11). This peptide contains 47% aspartic and glutamic residues, and because of the absence of homoserine or homoserine lactone presumably comes from the C terminal of HMG 1. The sequence of the first 44 residues of this peptide has been determined on a protein sequenator (unpublished results). As this sequence contains only 10 aspartic and glutamic residues (23%), the majority of the aspartic and glutamic residues must, therefore, be concentrated in the C-terminal portion of this peptide. We present here evidence that the aspartic and glutamic residues in the C-terminal region of this peptide are, in fact, present as a region of 41 aspartic and glutamic acid residues in continuous sequence.

The elution profile of a tryptic digest of peptide CB2 on a Sephadex G-25 column is shown in Fig. 1. The elution profile shows a peak (T) which elutes just within the excluded volume of the column. This peak was further purified by descending chromatography on Whatman 3MM paper in butanol-pyridine-acetic acid-water (90 : 60 : 18 : 72) for 7 d. A single peptide (T1) moved about 1 cm from the origin leaving a small amount of ninhydrin-positive material at the origin. This peptide (T1) was eluted with 5% acetic acid and lyophilised. The amino acid composition of peptide T1 was determined as Lys₃, Asx₁₂, Glx₂₉ and the N-terminal amino acid shown to be lysine by dansylation. The amino terminal sequence of this peptide was determined on a protein sequenator and was shown to be Lys-Lys-Glu-Glu-Glu-Glu-Asp-Glu-Glu-Asp-Asp-Glu. As this peptide was produced by tryptic cleavage of CB2, a lysine residue would be expected at the C terminal of this pep-

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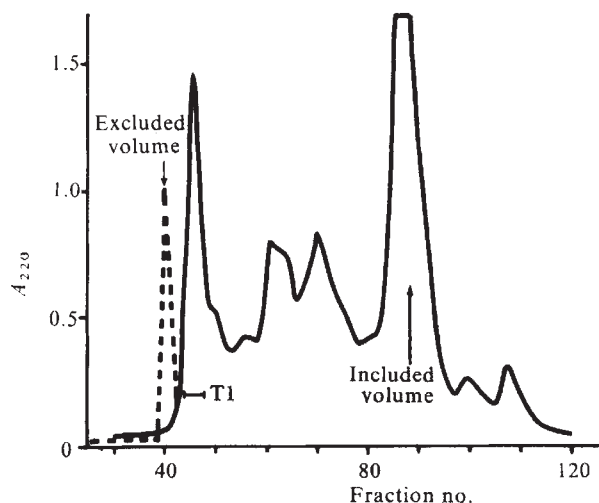


Fig. 1 Sephadex G-25 (90 × 2.4 cm) elution profile of the tryptic digest of CB2. For the digest, peptide CB2 was dissolved in 0.2 M ammonium bicarbonate (10 mg ml⁻¹) and incubated at 37 °C at an enzyme to peptide ratio of 1 : 50 (w/w) for 4 h. The digest was lyophilised and run in the column in 0.01 M HCl: 5 ml fractions were collected.

tide. This peptide could not end at the C terminal of HMG 1 as we have previously shown the C-terminal sequence of HMG 1 to be Phe-Ala-Lys¹². It therefore seems, from the amino acid composition of peptide T1, that we have a continuous sequence of 41 aspartic and glutamic residues with a lysine residue at each end. This peptide was taken through 25 cycles on the sequenator, and although unambiguous identification of the sequence beyond step 12 was not possible, no asparagine or glutamine residues were observed on any of the cycles. Additionally, it is unlikely that any of the aspartic or glutamic residues in this peptide are amidated, since we have previously shown¹² that only about 11 aspartic and glutamic residues are amidated in the total molecule, and we have found this number of amidated residues in other peptides during our sequence studies on this protein. The known difficulty of removing the thiazolinones of aspartic and glutamic acid from the sequenator cup at the butyl chloride wash step presumably contributed to the considerable amount of overlap that was evident by cycle 12. Both aspartic and glutamic acid residues were identified on the later cycles, but it was not possible to determine an unambiguous sequence.

We have previously shown that peptide CB2 does not bind to DNA¹¹, unlike total HMG 1. This is not surprising considering the acidic nature of this peptide. It seems likely, however, that this region will combine with basic parts of other chromosomal proteins and may even compete with the DNA and possibly break down the nucleosome structure. Interference with the DNA-histone interactions, or possibly some form of higher order structure, or cross-linking, would seem the most likely functions for this molecule, considering that there is only sufficient HMG 1 for approximately one in six nucleosomes⁶.

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Electron density map of apoferritin at 2.8-Å resolution

THE structure of ferritin is of considerable interest because of its widespread occurrence in higher organisms, signifying a general need to store iron and remove this essential, but toxic element. Horse spleen apoferritin has a molecular weight (MW) of about 444,000 and is composed of 24 subunits (MW 18,500) each containing about 163 amino acids¹. These are arranged in 432 symmetry to form a nearly spherical hollow shell with outside and inside diameters approximately 130 Å and 75 Å respectively^{1–3}. The large cavity inside the molecule can store up to 4,500 Fe atoms, packaged in a microcrystalline inorganic component of approximate composition (FeOOH)₈ (FeO:OPO₃H₂) (refs 4–6). The atomic structure of the micro-crystals is not specifically related to the surrounding protein structure⁶. Apoferritin catalyses the oxidation of Fe(II) which it retains inside the molecule as the ferric hydrolysate^{7–9}. Our 6-Å resolution structure of apoferritin³ showed the presence of several rods of electron density, tentatively assigned as α helices, and channels passing through the shell, which could provide an access route for Fe atoms. These features have been confirmed at 2.8-Å resolution and we can now also provide a plausible subunit conformation and quaternary structure.

Ferritin was purified from horse spleen and reduced with sodium dithionite at pH 4.8 (ref. 2). Apoferritin crystals (F432, *a* = 184 Å, *Z* = 4) were grown as previously described³. Two heavy atom derivatives used for phase determination were prepared by soaking crystals in 1% CdSO₄ solution containing either *p*-chloromercuribenzoate (PCMB) or K₃UO₂F₆.

Data to 2.8-Å resolution were collected on an Arndt-Wonacott oscillation camera using monochromatised Cu Kα radiation. Heavy atom parameters were refined using full matrix least squares refinement on centric data. Phases for all reflections were calculated by the method of Blow and Crick⁹ using anomalous scattering information¹⁰. The mean figure of merit for the phase set was 0.62. Details of heavy atoms derivatives used are shown in Table 1.

The electron density map, small portions of which are shown in Fig. 1, shows that the subunits are arranged within the

Table 1 Principal heavy atom parameters after refinement

Derivative	OCC†	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	<i>U</i> _{iso} (Å) ²
PCMB	0.488	0.1610	0.1783	0.1999	0.503
(<i>R</i> _c = 55%)*	0.271	0.1216	0.2130	0.0240	0.584
	0.089	0.0754	0.1234	0.2720	0.1‡
K ₃ UO ₂ F ₆	0.164	0.0154	0.1489	0.1738	0.433
	0.093	0.0640	0.0766	0.2156	1.086
(<i>R</i> _c = 62%)*	0.071	0.1136	0.1743	0.1692	1.49
	0.060	0.0150	0.1375	0.1875	0.5‡
	0.080	0.0500	0.0930	0.2190	0.5‡

*Crystallographic residual $R_c = \frac{\sum(|F_{PH} - F_P| - |F_H|)}{\sum|F_{PH} - F_P|}$

†Relative occupancy, at an arbitrary overall scale factor.

‡Parameter not refined.

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1. Bradbury, E. M. in *The Organization and Expression of the Eukaryotic Genome* 99–124 (Academic, New York, 1977).

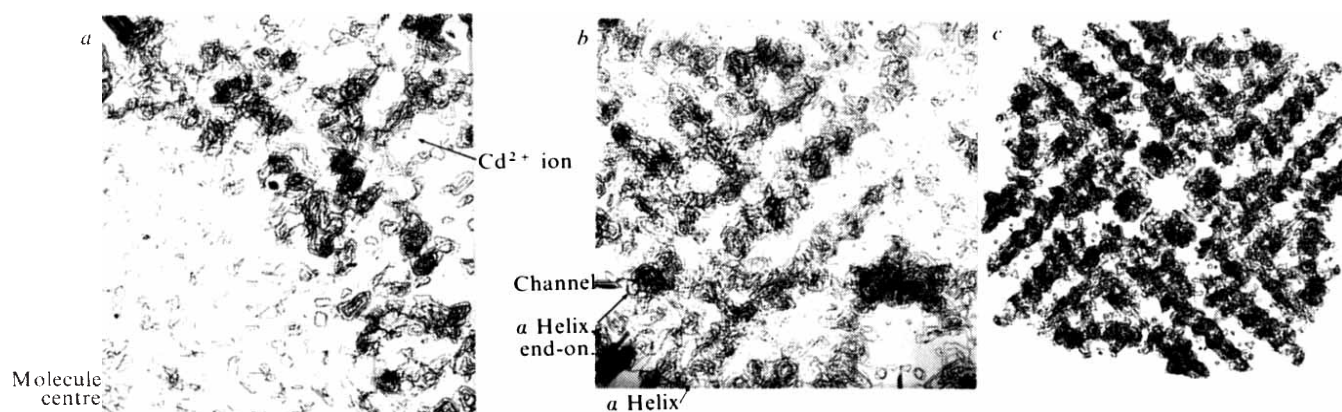


Fig. 1 Sections of the 2.8-Å resolution electron density map of horse spleen apoferritin viewed nearly parallel to the fourfold axis. Each part of the map shown comprises sections at intervals of $1/132$ of the unit cell edge, with contours at equal and arbitrary levels. A complete slice through the molecule is obtained by operation of the fourfold symmetry axis. *a*, Eight sections near the centre of the molecule. The molecule is seen as a hollow shell, two layers of helices thick. Two peaks related by a unit cell dyad (dotted line) form a double bridge between neighbouring molecules in the crystal. We attribute these to Cd^{2+} of crystallisation. The full circle near to the twofold axis, but on the inner surface of the shell shows the position found for a Tb^{3+} ion in a separate isomorphous replacement study. *b*, Nine sections near the top of the molecule. The left hand edge of the map cuts through one of the six channels which penetrate the shell along the fourfold axis. The subunits of the shell each comprise four long α helices (some shown in the illustration) and a short helix. The short helices from adjacent subunits surround the channels and can be seen on end. *c*, A complete slice about 100 Å across and 10 Å thick through the apoferritin electron density map near the top of the molecule viewed down a fourfold axis. Scale of (*a*) and (*b*) approximately $1.6 \times$ that of (*c*).

protein shell so as to form a bilayer of α helices enclosing the central cavity (the iron core in ferritin). The helical axes lie perpendicular to the radius vector, hence one layer of helices faces inwards and the other outwards.

Subunits are roughly cylindrical with h about 55 Å and d about 27 Å. The main structural features, accounting for about two-thirds of the primary structure, are four very long α helices (34–42 Å) with their axes nearly parallel, making up most of the cylinder length. There is an additional short length of helix perpendicular to the major helices. We have attempted to trace the complete course of the polypeptide chain in the electron density map and to determine its direction, but in the absence of sequence information, this cannot be done with complete confidence. However, although there are some ambiguities in inter-helical connectivities, which lie at the molecular surfaces, a plausible interpretation is shown in Fig. 2. In this structure the four long helices form two anti-parallel pairs. Helices B and C seem to be connected at their opposite ends by a long, extended loop, such that the pairs of helices are related by a pseudo-dyad perpendicular to their lengths. The short helix, E, probably lies at one of the chain termini.

We have tentatively assigned the N-terminus to the end of a

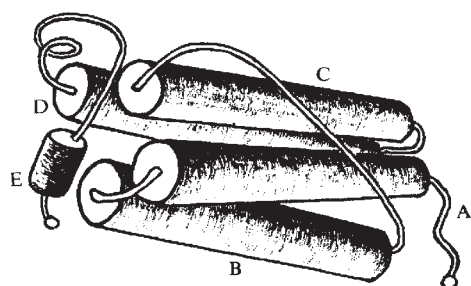
short non-helical segment attached to helix A. The long connecting loop between helices B and C lies over the surface of the apoferritin molecule. Proteolytic attack at this accessible loop could account for the 6,000–8,000 and 10,000–12,000 MW fragments sometimes observed on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate^{11,12}. The tertiary structure of a subunit superficially resembles myohaemerythrin¹³, which also contains two anti-parallel pairs of helices, and the tobacco mosaic virus (TMV) subunit¹⁴, but in TMV subunits pack with their helices lying radially rather than tangentially as in apoferritin.

The packing of subunits into the quaternary structure of apoferritin suggests the formation of stable dimers as intermediates in self-assembly. Subunits related by molecular dyads overlap along most of their length (Fig. 3*a*) with a larger mutual area of contact than those around threefold (Fig. 3*b*) or fourfold axes (Fig. 4*b*). Eight helices in the dimer lie roughly parallel to one another. Contacts between subunits around fourfold axes are relatively tenuous and stable symmetrical tetramers would not be expected (Fig. 4*b*). Operation of the fourfold axis produces approximately 10 Å-wide channels through the protein shell noted at low resolution³ (Fig. 4*a*, *b*), which are lined by short helices (E in Fig. 2), seen end-on in Fig. 1*b* and *c*.

Around the threefold axis the contact region is greater than at the fourfold. The cylinders form a three-blade propeller arrangement, with one end of the cylinder lying approximately perpendicular to the cylindrical surface of its symmetry-related neighbour (Fig. 3*b*). Formation of hexamers by aggregation of dimers around the trigonal axis might be expected to be a second stage in assembly. The subunit interactions are reminiscent of those in methaemerythrin, where parallel and perpendicular cylinder stacking is also found¹⁶, although the overall symmetry and number of inter-subunit contacts differ. A schematic drawing of the apoferritin quaternary structure viewed down a fourfold axis is shown in Fig. 4*a* in which it can be seen that each subunit is in contact with five neighbours.

Several metal binding sites can be located with respect to the subunit structure. The major PCMB site is near the threefold axis, towards the outside of the molecule. Other sites are near either the molecular or cavity surfaces. No metal ions have been found embedded within the shell either within or between subunits. Pairs of peaks, which we attribute to Cd^{2+} of crystallisation, occur close to the twofold axes on the outside of the molecule and connect adjacent molecules in the crystal

Fig. 2 Schematic drawing of an apoferritin subunit. The subunit contains four nearly parallel helices 34–42 Å long and a short helix, E, at right angles to these. Four of the short helices, E, line the channels while the long helices lie along the molecular surfaces. Non-helical segments connecting the helices are indicated. These have been derived from the map and provide a plausible subunit construction, although the connectivities cannot as yet be assigned with certainty.



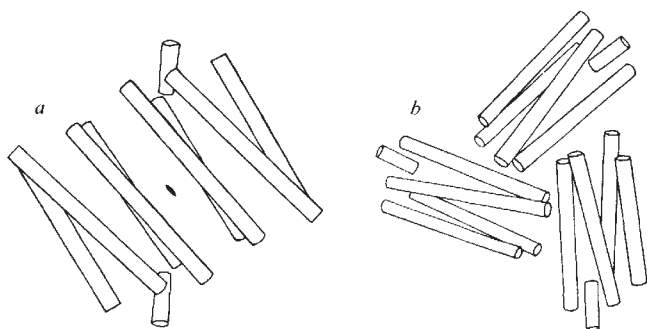
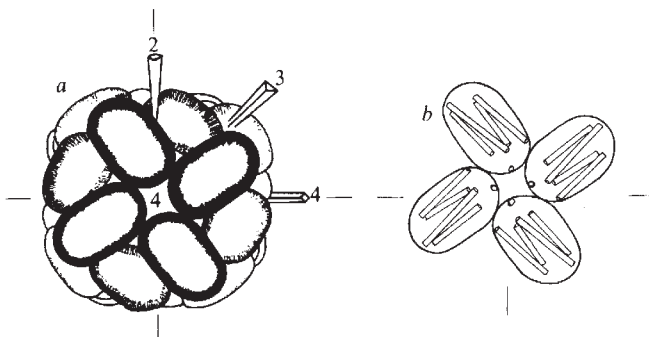


Fig. 3 Schematic views of apoferritin subunits, showing local packing around *a*, molecular twofold axes, *b*, molecular threefold axes. Only helical regions are shown. The helical rods from neighbouring subunits related by molecular dyads overlap along most of their lengths, and give a system of eight nearly parallel rods. A symmetrical dimer is thus suggested as a stable intermediate in the assembly of the protein shell. Probable interhelical connections suggest that chain directions alternate so that neighbouring helices are antiparallel. Subunits related by molecular threefold axes have their helices nearly perpendicular and there is less inter-subunit contact than around the twofold axes.

(Fig. 1*a*). The Cd^{2+} ion pairs are bound roughly half way along the extended BC loop. Four loops and two Cd^{2+} ions participate in the double intermolecular bridge.

Not used in the structure determination, but found in separate isomorphous replacement studies are Tb^{3+} ions on the inner surface of the shell. The major site (Fig. 1*a*) is close to the twofold axis at a radius of 41.6 Å and only 4.3 Å from its symmetry-related pair. The Tb^{3+} sites which probably involve carboxyl groups¹⁵, are of special relevance to ferritin formation. Our mechanism for the catalytic action of apoferritin in the oxidation of Fe(II) , which gives it the power to accumulate Fe(III) , becoming ferritin, involves sites for Fe(II) -oxidation and heteronucleation of hydrous ferric oxide⁸. Tb^{3+} ions inhibit iron uptake by apoferritin, and may bind at Fe-binding sites.

Fig. 4 Schematic drawings of the subunit arrangement in an apoferritin molecule viewed down a fourfold axis. *a*, Complete molecule showing some of its symmetry axes. *b*, Subunits related by a fourfold axis. Subunits approximate to cylinders or ellipsoids with length (approximately 55 Å) about twice their diameter (approximately 27 Å), although the shell is rather more smooth than these shapes suggest. Each subunit is in contact with five neighbours, although the contacts are of only three different types around the three different symmetry axes (as in Figs 4*b* and 3*a*, and 3*b*). The four major helices present in each subunit have their axes roughly parallel to the long axes of the subunits as drawn schematically in *b*. The interactions between subunits making the symmetrical tetramer seen in *b*, are less extensive than those in the symmetrical dimer and trimer of Fig. 3*a* and *b*. Compare with Fig. 1*c*.



The presence of a double site suggests the possibility of a cooperative two-electron transfer to oxygen from two close Fe(II) , giving two Fe(III) , which is followed by the formation of an oxo-bridge by elimination of protons from bound water molecules. Here again we can compare the structure with that of myohaemerythrin¹³, in which each subunit contains a pair of Fe atoms 3.44 ± 0.05 Å apart, which reversibly binds oxygen. This Fe-Fe pair is set well within the subunit, whereas in apoferritin the Tb^{3+} ions belong to different subunits and lie on the inner surface of the protein shell.

We thank the SRC and the Royal Society for financial support. We thank Drs H. Watson and H. Muirhead and their colleagues at Bristol for assistance and for providing facilities for X-ray film measurement and processing.

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Erratum

In the letter 'Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3' half and the 5' terminus of virion RNA' by P. Mellon & P. H. Duesberg, *Nature* **270**, 631, lines 32–35 of paragraph 4 should be deleted. In the last line of paragraph 4 the numbers 6 and 3 should be underlined.

Corrigendum

In the letter 'On melting icebergs' by H. E. Huppert & J. S. Turner, *Nature* **271**, 46, in the first line for 'their' read 'suitable'.

Nature Index and Binders

The complete **Index** for 1976 is available, price £2.50 (UK), US\$5.00 (Rest of World). Copies of the 1975 index are still on sale, price £2.25 (UK), US\$5.00 (Rest of World).

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reviews

Pulsar compilation

F. Graham Smith

Pulsars. By R. N. Manchester and J. H. Taylor. Pp. 281. (Freeman: San Francisco and Reading, 1977.) \$9.95.

THE ten years since the discovery of the pulsars have seen the publication of a bewildering flood of information on their properties, a plethora of theories about how they work, and a rather more coherent set of papers on their distances, their population within the Galaxy, and on the effects of the interstellar medium on the propagation of their radio pulses. Drs Manchester and Taylor have between them contributed a large proportion of the observational material now available to us, and it is very helpful to find so much assembled in one volume.

The difficulty with the pulsars is that we do not really know how they pulse; more precisely, we do not know how they produce the searchlight beam which crosses our line of sight each time the underlying neutron star rotates. The book cannot therefore start with an exposition of the structure of a pulsar, working from the core of the neutron star outwards, because it would be in difficulty as soon as it left the surface and entered the magnetosphere which is the seat of the observed phenomena. The authors therefore take the course of piling the observations into the first two-thirds of the book, leaving the reader dazzled with a rich assortment of phenomena, and presenting the theories in the remaining third. The theories have turned out to have almost the same richness and variety as the observations themselves. Here is the problem: should the authors select what they regard as the key observations, indicate those theories that can possibly accommodate them, and throw out the theories that now seem impossible? The alternative, which the authors adopt, is to keep an open mind and trust that the reader will be able to do the sorting out for himself. As a consequence, the origin of the pulses moves, from page to page, and even within a page, from near the surface to outside the velocity of light cylinder without much guidance to the reader.

The chapters on the interstellar medium and the population of pulsars are very useful and clear expositions. There are good discussions of the Crab

Nebula, and on the relativistic effects to be expected in observations of the binary pulsar. The chapter on X-ray pulsars is a useful introduction, although the subject grows so rapidly that it is already out of date.

The observational material includes some previously unpublished work, and is up-to-date in including the optical observations of the Vela Pulsar, made in January 1977. This latter work, incidentally, has been inserted into a

chapter on X-ray pulsars and binary systems, neither of which is an appropriate category.

Given that there is as yet no key to unlock the main mystery of the pulsars, this book is welcome as a compilation and an authoritative commentary. There is a good index to help one find one's way around. □

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Protein chemistry for immunologists

Immunochemistry of Proteins. Vols 1 and 2. Edited by M. Z. Atassi. Pp. 438 and 485. (Plenum: New York and London, 1977.) \$53.40 each volume.

THE first volume is a hotch-potch of a book, masquerading under a false title and falling between a definitive work and a collection of contributors' 'pet topics'. Over half of the book is devoted to chapters by the editor himself (M. Z. Atassi) and a colleague (A. F. S. A. Habeeb), on chemical modification and conformational aspects of proteins, respectively. And much of what they say could hardly be considered as immunochemistry according to the accepted meaning of the term. Habeeb does, however, make a few comparisons between the structural and antigenic relationships of proteins of different species of origin, besides offering some reflections about the application of immunochemistry which include the somewhat obvious conclusion that "... advances in immunochemical research in the last two decades resulted from the development of new techniques and methodologies".

The selection of the topics treated by most of the other authors has obviously been on an arbitrary basis. For instance, along with useful contributions on histocompatibility antigens (still very much in the throes of characterisation) and the antigenicity of collagen and encephalitogenic proteins, there is but one chapter dealing with the study of antigen-antibody interaction (by the somewhat specialised fluorescence polarisation technique). There is also a chapter on lymphoid cell responses to protein and peptides. All in all then a 'very mixed

bag' of subjects, of variable immunochemical appeal.

The recent appearance of a second volume in the series has done little to mollify these types of criticism. Again, there are useful practically orientated chapters, such as the one by Parikh and Cuatrecasas on affinity chromatography and other more theoretically inclined ones dealing with topics like "the effect of antigen structure on immunogenicity". There is also a chapter on Concanavalin A.

Over half of this volume, however, is devoted to contributions on the antigenic structures of those proteins which have now been well characterised, that is, myoglobin, lysozyme and tobacco mosaic virus. And, although the major contributions to this field by Atassi and his coworkers serve as valuable models to those engaged in the similar characterisation of other proteins, one cannot help gaining the impression that these two volumes are mainly outlets for wider propagation of such approaches to structural characterisation rather than attempts to provide well balanced texts on immunochemistry. Where, for example, is any treatment of the contribution of the antibody to immunochemical reactions, or of other ancillary proteins like the complement components?

Perhaps such topics will be considered in later volumes. In the meantime, a fairer title of the present two volumes would be *Protein Chemistry for Immunologists*, but even then the coverage is 'uneven' to say the least.

D. R. Stanworth

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Mathematical conversion kit

Mathematics Applied to Continuum Mechanics. By Lee A. Segel. Pp.xviii + 590. (Macmillan: London and New York, 1977.) £14.25.

ALTHOUGH the general tradition of mathematics departments in the US had been rather specialised towards pure mathematics, the best American universities have recognised for many years now the need for Applied Mathematics Programs. These have been aimed, especially, at helping those who, although brought up in the pure mathematics tradition, would like to consider the possibility of transferring their interest to fruitful areas of application of mathematics.

For example, one of the finest and oldest of the world's technological universities, Rensselaer Polytechnic Institute in Troy, New York, has given an introductory applied mathematics course to its mathematics students for tictity theory contributed by G. H. Handelman initiated it. It was later developed by Dr L. A. Segel, whose book *Mathematics Applied to Continuum Mechanics* in 12 chapters (with the material of chapters 4-6 on elasticity theory contributed by G. H. Handelman) has grown out of the course.

Applications of mathematics fall into two main areas. One involves the discernment and utilisation of laws of nature, as illustrated by Dr Segel's book or by its more elementary predecessor *Mathematics Applied to Deterministic Problems in the Natural Sciences* (C. C. Lin and L. A. Segel (Macmillan: London) A contrasting area of application is to statistical treatments of observed regularities and variabilities in systems where no underlying laws are known. Both are of immense practical utility, and it is important that mathematical education should make students aware of both.

In Britain, those taking mathematics in the immediate pre-university years have commonly had that advantage, being exposed both to mechanics and statistics as well as to pure mathematics, and their university course has built on that background and developed considerably within the area of mathematics applied to engineering and the natural sciences, as well as within statistics and operational research. More recently, in gross neglect of the country's needs, many schoolteachers have taken away from students the uniquely valuable example of elementary mechanics as an area illustrating mathematics applied to problems in engineering and the natural sciences, where natural laws are discerned and

utilised. Consequently, students enter university mathematics departments without any knowledge of this great division of applied mathematics, and with an educational background based on pure mathematics with some leavening of statistical distribution theory.

For the first time, then, British universities may be facing the problem which has long existed in the US, and which the best American universities have been seeking to counter with books like those of Dr Segel. Such books, directed rather explicitly at students with a good modern background in pure mathematics, but with little or no idea of classical applied mathematics or the uses of mathematics in engineering science, are aimed at capturing the interest of such students, and at bringing them to begin thinking in ways valuable to such applications.

Exercises abound throughout this excellent book and are effective (with or without use of the 'hints' section at the end) as a major method of exposition of material. Part A gives a good grounding in vector and tensor algebra and calculus, with particularly clear explanations of why all the definitions are appropriate. Part B gives a good introduction to rate-of-strain, viscosity, vorticity and boundary layers; and to strain, elastic constants, energy and uniqueness principles, bending, buckling, torsion, plane strain, generalised plane stress, and elastic waves.

Physical variability of the oceans

Variability of the Oceans. By A. S. Monin, V. M. Kamenkovich and V. G. Kort. Pp.xiii + 241. (Wiley-Interscience: New York and London, 1977.) £14.95; \$25.35.

THIS monograph by three well-known Soviet oceanographers is a survey of the physical variability of the oceans on widely varying space and timescales. Although the writing is clear and the errors few, I suspect that the book will have a limited readership.

The major shortcoming is that the most recent reference to the Western literature is 1973, and only 6% of all Western references are dated after 1970. Soviet citation runs a bit later, as one would expect. As any casual observer of physical oceanography will have noticed, ocean variability has probably been the area of greatest advance since 1970, and understanding of many parts of the spectrum has improved markedly since then. Furthermore, oceanography is a very technologically dependent subject, and it is in technology that the Soviet Union most obviously lags

Part C, on water waves, is concerned mainly with the deep-water case. There is a good introduction to group velocity. For example, the crest pattern is found not only for gravity waves generated by a large obstacle in steady motion (ship waves) but also for the limiting case of a very small obstacle making capillary waves. The author calls these 'beetle waves' in reference to a pretty frontispiece photograph! Part C concludes with a brief but interesting treatment of second-order effects. For me, only its opening chapter, deriving the free-surface condition and other basic equations, seemed to me to adopt an unnecessarily cumbersome approach based on excessive adherence to 'scaling' doctrines.

Finally, Part D is concerned with setting out the calculus of variations and its important applications in mechanics, and continues the excellent practice of getting the reader to work out the details in most of the really interesting examples. Wherever universities are faced with 'converting' students grounded primarily in pure mathematics to the great variety of its exciting and important applications in the mechanics of solids and the mechanics of fluids, I believe that they will find this book a most valuable conversion kit.

James Lighthill

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behind the West. Thus the more recent Soviet references do not make up for the dated discussion of Western work to the extent that one might otherwise have expected. The commonly remarked Soviet strength in theory does not compensate in this field for their missing observational strength, but does lend a formal tone to much of the book.

A good textbook need not be fully modern, and oceanography painfully lacks such textbooks. Students trying to enter oceanography, however, will find the subject matter here too terse. For those already conversant with the field, the obsolescence will be a serious defect. Perhaps the book is best used as an annotated bibliography of material through 1970. It also has historical value, as it provides insight into the thinking of some of the most prominent Soviet oceanographers in the early 1970s. In many ways, the book reads like the type of overall summary of a field that has become a commonplace in recent years as a preamble to and justification for large cooperative national and international field programmes. I suspect that is its origin.

Carl Wunsch

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Physical aspects of biological systems

Biophysik: Ein Lehrbuch. Edited by W. Hoppe, W. Lohmann, H. Markl and H. Ziegler. Pp. 720. (Springer: Berlin, Heidelberg and New York, 1977.) DM98; \$43.20.

A TEXTBOOK which describes biophysical approaches and physical aspects of biological systems would fill a gap, because most existing books deal with methods preferentially. The present book written by 54 authors, mostly from Germany, takes an interesting step in this direction.

The strongest part of the book is contained in the many chapters on biophysical aspects of various systems written by many well known workers in the field. These contributions range from enzymes as catalysts (Huber), membranes (Fisher, Stoeckenius, Sackmann and Frömter) and receptors (Thurm, Kaissling, Menzel, Snyder and Stieve), through photosynthesis (Renger) and muscle-contraction (Mannherz, Holmes) to complex systems such as information transduction in nerve systems (Creutzfeldt), orientation in flight (Reichardt), biomechanics of flight (Nachtigall) and transport of water in plants (Ziegler)—to mention only a few examples. Most of these contributions are very informative reviews of 10–20 pages written for undergraduate students or scientists who wish to broaden the scope of their knowledge. They provide a convenient source of information for many facets of biophysics and cannot be found in such a concise way in other textbooks. Three purely biological or biochemical chapters on the living cell (Schnepf), chemical structure of biological macromolecules (Tschesche) and biological function of nucleic acids (Zillig) are useful, but the information can be found also in other texts.

Chapters dealing with more general aspects of biophysics such as intra- and intermolecular interactions (Hofacker and Ladik), energy transfer (Dörr and Kuhn), cybernetics (Marko) or evolution (Kuhn and Schuster) do not add up to a complete picture. This part of the book naturally suffers more than the other part from the way it was written, namely by many rather specialised authors. For example, the chapter on interactions, written by two theoretical chemists, emphasises the quantum mechanical approach for the theoretical calculation of hydrogen bonds and charge transfer complexes but leaves out hydrophobic interactions and other important contributions.

Chapters on energetics and kinetics contain a basic sketch of general thermodynamics and fundamental kinetics without much emphasis on biological problems. Sections on thermodynamics of macromolecules in solution, multiple equilibria, polyelectrolytes, and so on, would have been valuable. Surprisingly the dynamic aspect of the function of biological macromolecules are not treated, although a good section on fast kinetic methods (Rüppel) is included.

A chapter on physical methods for the investigation of biological macromolecules is of heterogeneous quality. The description of methods for solutions cannot compete with good intro-

ductionary texts such as Van Holde's *Biophysical Chemistry*.

Lecturers and students who are engaged in courses of biophysics would welcome a text on general biophysics. The present book cannot be recommended as a basic text but it may serve as a supplementary book for advanced reading. A difficulty for a worldwide use of the book arises from the fact that it is written in German. This is an anachronism for a book of this type, but perhaps an English translation is planned.

Jürgen Engel

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Indignation against mercury pollution

Mercury Contamination: A Human Tragedy. By P. A. D'Itri and F. M. D'Itri. Pp. xxii+311. (Wiley: New York, London, Sydney and Toronto, 1977.) £13.45; \$22.80.

THE aim of this book is to raise indignation against environmental pollution in general, and pollution by mercury in particular. Beyond the problem of environmental pollution, there is an historical account on the use of mercury. The story of its medicinal use, especially for the treatment of syphilis (as presented by the authors), is a warning to scientists to analyse all information critically and not to substitute possibilities for facts. Unfortunately, the authors do not draw the same conclusion.

There is no doubt about the environmental impact of mercury pollution in the North American lakes and rivers; there is no doubt about the unacceptably high level of methylmercury in fish caught in some of these waters; there is no doubt that methylmercury is a highly toxic accumulative poison, but these facts are not substitutes for the complete lack of controlled morbidity data for the populations of White Dog and Grassy Narrows Reserves. The lack of morbidity or mortality data, to say the least, is surprising. It is alleged that an official admitted "it was a wonder more Ojibways (of the Grassy Narrows) were not dying because some of them had blood mercury levels that exceeded those of fishermen who died at Minamata". It is also surprising that these Indians had higher blood levels than fishermen at Minamata, as, according to the authors, the highest hair level of the members of the Ojibway tribe was 40% lower than the hair level at which Japanese at Minamata began to show

symptoms. Another reason for surprise is that blood mercury concentrations were not estimated in the victims of the Minamata epidemic. In the same chapter there is another misquotation or uncritical quotation which states that 200 g fish consumed for 3 weeks raises the mercury concentration in the blood to 2 p.p.m. A 70-kg man, however, ought to consume 10.7 mg mercury as methylmercury per day for 3 weeks to produce 2 p.p.m. blood concentration—that is, he should eat fish containing an unbelievably high (more than 50 p.p.m.) concentration of mercury.

These and similar overstatements do not help but rather weaken the impact of the book. Mercury, according to the authors, injures at a lower dose and at an earlier time than scientific evidence indicates. As they suspect that mercury had some role in the death of Napoleon, they bring forward his death before the One Hundred Days and Waterloo to the time of his first exile on the Isle of Elba.

These criticism might seem unjustified, as in the preface the authors made it clear that "The book was not written for scientists who conduct research into mercury pollution", probably because compared with the *Toronto Globe and Mail*, and the *Kenora Daily Miner and News*, the scientists are inferior as creators and absorbers of information. First of all, "The scientific method by definition is partial and incomplete" (which is true); secondly, "local citizens instinctively grasp the problem and insist on resolution while scientists are still trying to ensure that their findings are accurate"; and thirdly, even if they are able to grasp the problem "the scientists carefully qualified statements may confuse the public."

Laszlo Magos

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Quantum pharmacology

Quantum Pharmacology. By W. G. Richards. Pp. xiii+213. (Butterworth: Sevenoaks, UK, 1977.) £12.

THE relationship between the chemical structure of small molecules and their biological effects has been studied intensively for over a hundred years. Apart from its intrinsic interest, it is obviously of enormous practical importance in the design of drugs. In recent years, studies of this relationship have been growing steadily in sophistication. The biological effects can now be described in greater detail, often in terms of binding to isolatable receptors and enzymes rather than gross physiological effects. The structural properties of the small molecules, too, are now discussed with greater precision, considering their three-dimensional conformation and electron distribution rather than simply two-dimensional representations of their covalent structure. In the latter area, the past ten years have seen increasing application of molecular orbital methods to molecules of pharmacological interest, and Dr Richards, one of the leading practitioners of this interdisciplinary art, has now written an introduction to the field.

The stated aim of the book is twofold, to introduce medicinal chemists to molecular orbital calculations and to introduce theoreticians to the pharmacological problems to which their methods can be applied. Thus, the book begins with an outline (in 87 pages) of some basic pharmacology, lucidly presented though necessarily very condensed. The emphasis is primarily on the cholinergic and adrenergic systems, histamine and centrally acting drugs, with chemotherapy, prostaglandins and peptide hormones, for example, receiving only brief mention.

The second part (49 pages) introduces molecular orbital calculations. The fundamental properties of wave functions and molecular orbitals are described very clearly, as is their use to calculate molecular properties. There is a valuable chapter in which the various approximate wave functions are described and compared.

In many ways the core of the book is the third section (38 pages), as it is here that the applications of theory to experiment are discussed. The first topic is that of the conformation of the small molecule, illustrated by calculations on a number of neurotransmitters and, in particular, on a series of histamine derivatives. The latter illustrate the "essential conformation approach" developed by the author, in which the biological activity of a series of compounds is correlated with the region of conformational space which they can occupy, and a region of this space which

is in some sense essential for activity is thus defined. Since most conformational calculations consider the small molecule in isolation (*in vacuo*), the next chapter is devoted to a discussion of approaches to the problem of including the effects of solvent (specifically water) in the calculations. The remaining chapters focus on electron distribution—both on empirical correlations with activity, and on the use of the electrostatic potential field of the small molecule as a method of "mapping" the complementary surface of the receptor site (introduced by Weinstein and his colleagues). It is a pity that this section of the book is so brief. It would have been valuable to have a more extensive discussion of some of the many interesting points raised, perhaps at the expense of a shorter pharmacological introduction, for which many introductory texts are available. The book concludes with a very valuable annotated bibliography of all molecular orbital studies of molecules of pharmacological interest reported up to the end of 1976.

Can calculations be useful? The author concludes with a cautious "yes", with which I am sure most readers will agree. The ways in which they are best used remains, to some extent, to be established. There is no question that molecular orbital calculations have revealed interesting correlations between molecular properties and biological activity—as, for example, in the "essential conformation approach" to histamine analogues—and these will continue to be a valuable aid to the rational design of new compounds. What we do not know is how such correlations can be interpreted in physical terms to help build up a picture of the receptor site. There is no doubt that further work in this field will be stimulated and guided by Dr Richards' lucid and critical description of the state of the art.

Gordon C. K. Roberts

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Remote sensing techniques

Aerial Photography and Remote Sensing for Soil Survey. By L. P. White. Pp. viii+104 (Clarendon/Oxford University: Oxford, 1977.) £6.

THE foreword to this book indicates that it is the first of a series of new handbooks following in the footsteps of Robin Clarke's classic *The Study of the Soil in the Field*. This is a disappointing book, mainly because it does not fulfil the expectations aroused by the title and preface. In short, the balance of the book seems to be out of keeping with the topic. Only one chapter (some eleven pages) is directly concerned with soil survey and aerial photography. The remainder of the main text of 93 pages consists of a concise discussion of remote sensing methods, sensors and platforms, which other texts have dealt with in a more comprehensive manner.

Readers concerned with the use of remote sensing techniques in soil survey will turn their attention to chapter 4. Those familiar with image interpretation will doubtless recognise the wide range of issues touched on here. The brevity of the treatment, however, results in a failure to discuss in any depth the many aspects of the relationships between soil mapping units and image characteristics. For example, the treatment of terrain analysis is rather perfunctory, and the topics of pedo-

logical analysis and photo-interpretation keys merit further discussion. A much fuller treatment of the value of images/photographs for the detection of temporal variations in soil conditions (for example, structure and profile drainage) would have been useful. Similarly, more attention could have been given to definitions of soil mapping units, their relationships to different scales of imagery, and the testing of the accuracy of interpretation.

The bulk of the book comprises descriptions of photography and photographic products, line-scanners, side looking radar, imagery from space platforms, image enhancement, and automatic analysis. There is, however, little significant comment on the sensors, platforms and techniques in relation to soil survey. Indeed, the illustrative plates have captions dealing with vegetation, relief and land use, rather than soil conditions or soil boundaries. Some plates are in colour.

A wider selection of references would have been appropriate in a volume of this kind. For example, the work of Beckett, Evans, Mitchell and Western would suggest some of the pathways towards greater use of remote sensing.

The line diagrams are good but there are some typographical errors such as incorrect naming of the general editors on the dust jacket.

L. F. Curtis

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what's new—lasers, optics, holography

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. More detailed information may be obtained by circling the appropriate numbers on the Reader Enquiry Card, bound inside the cover. A feature on centrifuges and rotary evaporators will appear in the 16 February issue.

Coherent. The Coherent Supergraphite series of argon and krypton ion lasers comprises 13 different models with 40 different configurations, providing multiline output power of from 800 mW to over 18 W TEM₀₀. With the ultraviolet option these systems will deliver up to 3.0 W guaranteed in the ultraviolet. Coherent krypton lasers have guaranteed TEM₀₀ output as high as 4.6 W in red wavelengths 3.0 W in the violet and 2.0 W in the ultraviolet. The Coherent CR series of dye lasers features a crystalline quartz birefringent filter which combines extremely low optical loss with precise tuning. Linewidth is resettable and the whole system is designed for ease of use. Optical components are mounted in a 2-inch diameter Invar rod for maximum amplitude and frequency stability. The CR 599 scanning dye laser system incorporates active closed-loop stabilisation to provide narrow linewidth and stable operation. The Coherent picosecond pulse package is based on Coherent ion and dye lasers and offers controlled pulse width and peak power, while the picosecond pulse picker ensures repetition rates. The new Model 307 'Noise Eater' can stand alone in front of any laser and give a



Laser equipment from Coherent

reduction of 40 db/d.c. and 26 db/1 MHz. Other Coherent equipment available includes collimators and lenses, mode lockers, interferometers, modulators, power meters and optics.

Circle No. 25 on Reader Enquiry Card

Korad. Korad manufacture holographic laser systems for both reflection and transmission holography. Features include long coherence lengths, excellent wavelength repeatability, and availability as single and double pulse systems. The Korad mobile holocamera is completely self-contained and weighs less than 200 lb. The holocamera can accommodate any of the more than 20 different laser systems now available. The Pockels cell Q-switch KQS2 and KQS2DP laser



Korad KHC1 holocamera

systems provide a precisely controlled laser pulse and are particularly suited to applications involving plasma and shock phenomena. Korad offer an extensive range of holographic accessories; these include alignment and reconstruction systems, the KA autocollimator, the KD1 photodetector, KDG delay generator and the 99/102C energy measurement system.

Circle No. 26 on Reader Enquiry Card

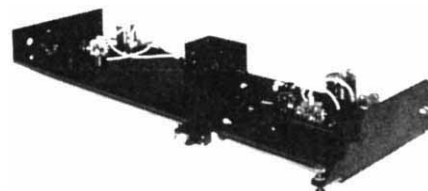
Hartley Measurements. The model 215G grating-tuned TEA CO₂ laser is a compact high energy density system: the 2-J multimode output has a gain switched spike only 40 ns wide, providing a peak power in excess of 15 MW. For TEM operation 0.7 J can be obtained in a 6-mm diameter beam of 3 milliradians divergence. Repetition rate is continuously adjustable from 0.2 to 2 ps. Manual push button/single shot firing are also available as standard. Full optics are provided with the unit and tuning is by means of a grating of 150 lines per mm. The unit is completely self-contained with a built-in power supply and all controls mounted in the laser housing.

Circle No. 27 on Reader Enquiry Card

J K Lasers produce the System 2000 range of pulsed solid-state laser equipment. An updated set of data is available to explain the system, and

applications covered include holography, phytochemistry, plasma diagnostics, welding and drilling. The J K Lasers range of pulsed ruby lasers designed for use in holography has been extended to include a 10-J single-mode system. This high output energy has been achieved by two-stage amplification of a well-proven holographic oscillator. The system is capable of taking holograms of objects within a 5-m cube. As 1 J of the output energy can be used as the reference beam, large-format holograms of up to 1 m² can be made from a single pulse. J K Lasers will shortly be offering a range of standard dye lasers suitable for pumping with their solid state lasers. These models will be available with a full range of dyes, operating throughout the spectral range 400–800 nm.

Circle No. 28 on Reader Enquiry Card



J K Lasers 2000 pulsed ruby laser

Electro-Photonics. The Electro-Photonics range includes a comprehensive range of flashlamp pumped dye laser systems, wavelength tunable across the entire visible spectrum, with options of mode-locking to produce picosecond laser pulses and second harmonic generation to provide tunable laser radiation in the ultraviolet. One interesting application is a new technique of 'rapid optical sampling of relaxation phenomena, involving two time-correlated picosecond pulse trains'. Two flashlamp-pumped, mode-locked and optically coupled dye lasers produce trains of picosecond pulses with definite, but variable time correlation. One pulse train is used to bleach the sample, while the other probes the decay of its transient transmission. Relaxation times between 15 ps and 1 ns can be monitored by one simultaneous activation of the two lasers that is, a single shot technique. In previous experiments each point of the relaxation decay curve was measured by splitting one laser pulse train and mechanically varying the delay between

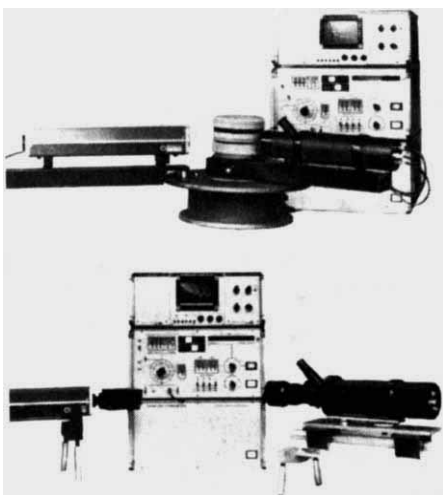
excitation and probe pulses between laser firings. There was also an added restriction that transmission measurements were limited to the probe wavelength. In this new technique, the probe wavelength can be tuned over a considerable wavelength range. Electro-Photonics also have developed systems for remote sensing of atmospheric pollution, in particular SO_2 and NO_2 . The technique used is differential absorption. The Electro-Photonics range includes the Model 23 high repetition rate dye laser, the Model 33 mode-locked dye laser and the Model 43 high-energy dye laser.

Circle No. 29 on Reader Enquiry Card

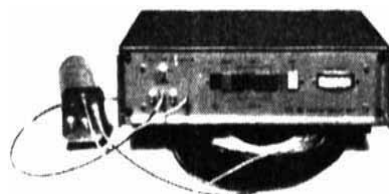
Malvern Instruments. The Malvern ST 1800 particle and droplet size distribution analyser functions over the range 1–500 μm . The analyser depends on the principle of laser-illuminated Fraunhofer diffraction scattering: the diffraction pattern generated by each particle is independent of its position in the beam, so measurements can be made with a particle moving at any speed. For a group of particles or droplets the combined diffraction pattern is uniquely related to the size distribution. The output from the detector system is processed to provide hard-copy paper print readout of percentage and cumulative weight distribution, and of the degree of obscuration. Particles in the size range 25 $\text{\AA}$ –1 μm can be sized by use of the System 4300 photon correlation spectrometer. Particles moving in a laser beam under Brownian motion yield, when examined by photon correlation, their diffusion coefficients and hence by Stokes-Einstein's equation, the hydrodynamic radius. Polymers, surfactants, colloids, micelles, emulsions, proteins and RNA molecules can all be studied in this way. The Malvern Type 6200 laser photon correlation anemometer measures flow velocities and turbulence.

Circle No. 30 on Reader Enquiry Card

Malvern 4300 (top) and 6200 systems



Electro Optic Developments. Products from Electro Optics include sub-nano-second rise time Pockels cells, wideband low voltage video modulators, phase modulators for laser anemometry, optical harmonic generators, high voltage amplifiers and pulse generators. The standard optical components offered include calcite polarisers and quartz retardation plates. Five years of active development work on wideband interference-free optical data telemetry links have culminated in the FOL 100 fibre optic link. This 150 MHz base bandwidth system features a compact,



Electro Optics FOL 100

rugged transmitter powered by rechargeable NiCd batteries. The transmitter sensitivity can be remotely selected at the receiver by means of a control fibre running parallel to the signal fibre. Transmission distance is 50m for 43dB SNR or 200m for 40dB SNR.

Circle No. 31 on Reader Enquiry Card

Combined Optical Industries. Combined Optical Industries of Slough offer a wide range of top quality magnifiers manufactured from optical grade plastics material.

Circle No. 32 on Reader Enquiry Card

Laser Lines. High peak powers at repetition rates of up to 100 Hz can be generated when a nitrogen laser is used as a pump. The self-contained Laser Lines Molelectron nitrogen laser can be used in this way as a pump for the new Molelectron DLII series of dye lasers to give complete coverage from 360 to 930 nm. These dye lasers all incorporate a new four-beam expander which is completely achromatic and expands the beam only at right angles to the rulings of the grating. This enables the standard laser to have a linewidth of 0.01 nm at 460 nm which can be reduced to 0.0006 nm when an air-spaced etalon is placed in the laser cavity. The DLII laser systems have an oscillator amplifier configuration. Only 25% of the pump beam is used in the laser oscillator, thus avoiding saturation effects, and the remainder is used in the amplifier. These systems can be used in conventional absorption and fluorescent emission spectroscopy, and the high powers and narrow linewidth enable more difficult techniques such as Raman and coherent anti-stokes Raman scattering (CARS) to be used.

Circle No. 33 on Reader Enquiry Card

Hakuto International. Recent additions to the HTV range of photomultiplier tubes are designed specifically for use in conjunction with helium neon lasers operating in the range 185–850 nm. Various types of head-on and side-on photomultipliers are available, ranging in size from $\frac{1}{2}$ to 5 inches in diameter, with spectral response up to 930 nm.

Circle No. 34 on Reader Enquiry Card

OCLI Optical Coatings. OCLI specialise in optical thin film coatings in laser systems. Increased use of lasers has introduced the additional hazard of eye damage by laser beams entering visual viewing sighting systems. OCLI Europe have developed a durable coating that can be applied to any surface in the optical system without requiring optical redesign, to provide protection against ultraviolet ruby and yag lasers simultaneously, whilst maintaining good visual transmission. OCLI also supply coated optics specifically designed for use in laser fusion and other advanced systems resulting in power densities greater than conventional coatings can withstand. These include windows, mirrors and polarisers.

Circle No. 35 on Reader Enquiry Card

Carl Zeiss Inc. The new Zeiss flatfield, long-working distance Epiplan microscope objectives are for brightfield and darkfield work. They are highly effective for manipulation under the microscope, offering extra protection against damage because of the long working distance (minimum 2 mm).

Circle No. 36 on Reader Enquiry Card



Zeiss Epiplan objectives

Macam Photometrics. The Macam Modern Optics Educational System has been specially designed to teach a wide range of physical optics techniques, including laser optics, diffraction, interferometry and the very versatile technique of holography. Macam also produce a range of photometers for the accurate measurement of the luminous flux of a wide range of lamps.

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